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THE ROLE OF THE SENSE ORGANS IN AGGREGATIONS OF *AMEIURUS MELAS*

EDITH S. BOWEN

AN EXPERIMENTAL STUDY OF THE WATER RELATIONS OF SEEDLING CONIFERS WITH SPECIAL REFERENCE TO WILTING

ROBERT MARSHALL

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THE ROLE OF THE SENSE ORGANS IN AGGREGATIONS OF *AMEIURUS MELAS*

BY

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THE ROLE OF THE SENSE ORGANS IN AGGREGATIONS OF *AMEIURUS MELAS*[1]

Introduction

The young of the siluroid fishes, the catfishes and bullheads, exhibit a striking type of aggregation which by its apparent simplicity offers a tempting study to one interested in the fundamental bases of group behavior in animals. In the species *Ameiurus melas* (Rafinesque), used in this work, the young, when observed in the summer months in ditches and small ponds, are usually in a close cluster, swimming slowly near the surface, and packed together into a more or less spherical mass so that individuals are frequently in contact. A single fortunate dip with a net has been known to take five hundred, even when a large part of the group was seen to escape. Sometimes in shallow ponds on a warm sunny day large numbers may be seen resting at the surface in close proximity to one another. When disturbed they quickly dart down into the water, and scatter in all directions. In the latter part of the season the groups are often fairly small, sometimes consisting of a dozen individuals or less.

If a group in a small ditch is scattered its reformation may be observed. Several minutes after the disappearance of the fish two or three individuals appear singly and come together into a region somewhere near the original location of the group. Gradually they are joined by single individuals or small groups which come, apparently by random swimming, into the same locality. These smaller groups take no definite direction toward the larger one until they are within a short distance, about two or three feet. Then they swim directly toward the larger aggregation and join it. This larger group continues to attract others until it has reached its original size. This procedure took from half an hour to an hour in the cases observed. The fishes always appeared from the muddy bottom singly or at most in small groups.

When brought into the laboratory, the young aggregated for several days in the aquarium. Three hundred or more would collect in a cluster three or four inches in diameter, usually against the side of the aquarium. When undisturbed for some time they would frequently be more or less scattered, but with a slight disturbance would collect again. An unusual disturbance would always scatter the group as in nature. After some time in the laboratory the aggregations grew less frequent, but could always be produced by striking the aquarium. During the winter the aggregations lost their com-

[1] This problem was undertaken at the suggestion of Dr. W. C. Allee whose stimulating interest and helpful suggestions have been deeply appreciated. The present investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

pactness; individuals were more frequently isolated, but usually aggregated when active. By strong stimulation, as in some of the experiments where the animals were placed beneath a bright light, aggregations could be induced at any time.

There has never been observed an indication of any specialization of function among the members of an aggregation. Leadership always falls to the fish which happens to be in the van. When the group turns, even slightly, leadership changes to the individual farthest in that direction. Two schools mixed readily when near together, and in the laboratory aggregations were formed when the largest fishes were three times the size of the smallest. The location of individuals in a school continually changes, but the form of the group itself remains much the same.

The breeding behavior of these fishes has been known since the time of Aristotle who observed it in the "Glanis." According to his description the eggs were laid among the roots of trees, and then the female, having spawned, departed, while the male watched and protected the young for forty or fifty days, until the young were sufficiently grown to escape from other fishes. Agassiz (1856), identified the "Glanis" as a siluroid closely related to our catfish and bullheads, and described similar behavior for Pomotis and Pimelodus in this country. Similar observations reported by others have been reviewed by Kendall (1910). The only reference to *Ameiurus melas* found so far in the literature is that by Fowler (1917), in which he gives a description, taken from notes by Dr. Van Dorn, of a pair spawning in an aquarium:

"Both sexes assumed charge of the nest and both guarded the young. Both parents remained in the nest at the same time, and at alternate times. The female was apparently the more savage and the male would bite one's finger when disturbed. The nest was a shallow depression, scooped out of the bottom, and its diameter about equal to the fish's length. The eggs, when deposited, were 200, and of a pale, creamy-yellow color. As the water was warm, they hatched in five days."

The sense organs of Ameiurus and their part in the behavior of individual fishes has been studied by several investigators. Herrick (1902), showed that in *Ameiurus melas* taste buds are present on the skin over the whole body, being especially abundant on the barblets, and that practically the whole cutaneous surface is sensitive to both tactile and gustatory stimuli. The olfactory organs were shown by Parker (1910), who worked with *Ameiurus nebulosus* (Le Sueur), to be distance receptors of chemical stimuli, and distinct from the chemical organs in the skin which are taste organs. He concludes that the senses of taste and smell differ in degree rather than in kind. According to Parker and Van Heusen (1917), Ameiurus is stimulated by metallic rods, probably through the gustatory organs. They also, (1917a), demonstrated that the ear, skin, and lateral line organs are all sensitive to vibrations. The

ear responds to wave lengths of greatest frequency, the lateral line organs to intermediate wave lengths, and the skin to the slowest vibrations. They found that the responses to the stimulation of the ear and of the skin are generally locomotor; those of the lateral line organs, inhibitory to movement.

While the eyes are probably of little importance in feeding, (Herrick, 1902; Parker, 1910; and Olmstead, 1918), Schiche (1921), from his study of the individual behavior of *Ameiurus nebulosus,* believes that the eyes are the controlling sense organs in the decidedly negative reaction to light, and in the orientation toward light. He states that optic sensitivity must be concerned in the control of movement since blind fish may remain in constant motion for a day at a time. A marked thigmotactic reaction is exhibited by these fishes, resulting in resting positions which bring as many points of the body as possible in contact with solid objects. This is illustrated by the "living-holes" or depressions which they make in the substratum, and to which they return at intervals. When the fish are subjected to conflicting stimuli, the chemical stimulus is the strongest, while thigmotactic and phototactic stimuli result in a response intermediate between those called forth by the separate stimuli.

In regard to the function of the sense organs in the aggregation of young fish, Eddy (1925), reported that blinded fishes do not aggregate. At that time he gave results, upon the effect of drugs and (1926) upon determinations of oxygen consumption which indicate that aggregating behavior is correlated with a relatively high metabolic rate.

Parr (1927), in a study of schools of typical pelagic fishes, reaches the conclusion that the behavior of individuals is controlled by visual stimulation alone. He believes, however, the catfishes (*Ameiurus*) are probably exceptional in this respect and that in their schools, which are globular and very dense, schooling and milling are dominatingly controlled by tactile stimulations.

The present work has been done in an attempt to analyze more fully the roles played by the different sense organs in the aggregations of catfishes.

Methods

The fishes used in this investigation were of the species *Ameiurus melas* (Rafinesque), and ranged in length from two to five centimeters. Two different types of experimental procedure have been employed. The first method was that of "preference" experiments carried out by means of gradient tanks. Here normal fishes were used in most cases, and the attempt was made to discover to what kinds of stimuli they gave a positive or negative reaction. In the second group of experiments the different sense organs were destroyed or rendered temporarily inactive, and the reactions of animals in a group were studied. The results of these two groups of experiments will be given separately and the evidence from the two methods discussed later.

Gradient Tank Experiments

Two different types of gradient tanks were employed in these experiments, but the principle was the same in both. A single fish placed in a long trough was allowed to swim back and forth, while the conditions introduced from the two ends could be made to vary in one particular only. The amount of time spent on the two sides of the trough was recorded by means of two stop watches. One measured the total duration of the experiment, 15 minutes in each case, while the other recorded the time spent on one side, *e.g.* the left. The time on the right side was then found by subtraction. The path of the fish was also recorded. In the great majority of the cases the fish was in motion most of the time, swimming back and forth or pushing against the ends of the tank. Since fishes varied in their individual tendencies to stay on one side or the other, a control for each experiment was run where the conditions were the same on both sides of the trough. Each series consisted of ten experiments. The percentages of the time spent in the same end of the experiment and the control were compared, and for both the experiment and the control

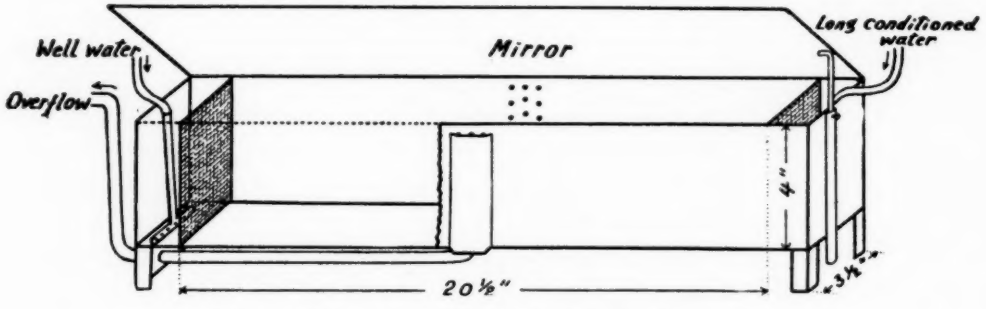

Fig. 1. Tank A, gradient tank for running water.

the percentage of time for one end was compared with the the fifty percent expected from random movements. The mean difference for each comparison is given with its statistical significance as calculated according to Student's Method. Values of .01 or below, indicating one chance or less in one hundred of as great variation from random sampling, are considered significant, those above .01 not significant.

Tank A (Figure 1) was arranged so that a slow current of water, three to four liters in fifteen minutes, entered at each end, and left through holes at the middle of the trough leading into an outlet pipe. The level of the opening of this pipe was adjustable and regulated the level of the water in the trough. The tank, make of galvanized iron, contained a wire screen at each end, and a wide meshed wire lining (not shown in the diagram) standing ½ in. from the sides of the tank to prevent the fish from coming into direct contact with the inlets and outlets. A mirror was arranged at an angle over the tank so that the image from the inside was reflected to the side and down, and the fish could be watched without the observer bending over the tank. The water

was siphoned from pans elevated about three feet above the tank, and entered the tank through two small brass pipes containing five openings and placed horizontally, one at each end. The tank was first filled with control water and then the current was started at both ends simultaneously, and at the same rate by the regulation of clamps. When colored water was employed a distinct line of division was observed in a few minutes at the middle of the tank with clear water at one end and colored water at the other. This tank was designed by Dr. Allee who suggested its use in this work.

The experiments were run in a dark room with two lights of the same intensity (40 or 50 watts) placed above and to the side of the tank, one on each side opposite the halfway point between the middle and the end of the trough. Thus the lighting was the same for both ends.

Tank B was simpler, consisting of a long trough of galvanized iron, 3 ft. long, 3½ in. wide, and 4 in. deep, with a mirror attached as in Tank A. The inside of this tank was covered with paraffin to prevent any action of metals, as Parker and Van Heusen (1917), have shown *Ameiurus nebulosus* to be stimulated by the dissociation of metals in water. This precaution was not feasible in Tank A because of the numerous small openings, but it is doubtful if this interfered to any extent with the results since the amount of metal on both sides was the same. The behavior of the fishes did not differ noticeably in the two tanks. In Tank B movable partitions could be placed at desired distances from the ends so as to give as long a gradient as possible and leave room at one end for a group of fishes. Only one 50 watt light placed opposite the middle of the tank was needed for lighting.

Well water used for these experiments was always allowed to stand overnight in large pans. This brought the oxygen content, as shown by Winkler's method, and the temperature to that of the aquaria. pH, which ran about 7.5—7.6 in the well water, was brought to the same point as that in the aquaria by adding a few drops of concentrated NaOH. The pH of the aquaria varied from 8.0—8.4 depending upon the length of time since well water had been added.

Series I and II. Tank A was used with clear conditioned water entering at one end, and well water colored with methylene blue entering at the other. The dye was added to permit observation of the currents. In the controls clear well water was run against colored well water. The conditioned water for this experiment had stood in the aquarium for several months, and had contained a variety of animals besides catfish. It was therefore water which had been highly conditioned heterotypically. The pH ran up to 8.4, which rarely occurred in other experiments. In five sets of each series the conditioned water was introduced from the left, in five from the right, to offset any tendency of the fishes to stay in one end. In the same way the order of the experiment and the control was varied with a rest of approximately 30 minutes between to offset any possible effect of a conditioned automatism

since catfishes tend to form place associations (Schiche, 1921). The fishes were from the same tank from which the conditioned water was taken.

When the percentage of time spent in the conditioned end of the experiment was compared with the percentage of time spent in the same end of the control, there was found to be a mean difference of 29.6% in favor of the conditioned water, with a significance of .0006. When, however, the time spent in one end of the control was compared with the 50% expected from random movements, there was found to be a mean difference of 28.2% in favor of the colored end with a significance of .000,000,1-. There is therefore a decidedly positive reaction to the colored water which agrees with the negative phototactic reaction of *Ameiurus* as described by Schiche (1921). In the experiment the factors of color and conditioning counteract each other and give no significance, but a definite tendency toward the conditioned water is shown by the comparison of the experiment and the control.

Series III and IV. These two series were the same as I and II except that no color was used. When the conditioned end was compared with the same end in the control there was found to be a mean difference of 22.15% in favor of the conditioned end with a significance of .0004. The experiments gave a mean difference of 11.65% in favor of the conditioned end with a significance of .005. These results indicate a positive reaction to highly conditioned water as against unconditioned well water by fish adapted to this amount of conditioning.

Series XI and XII. In these experiments well water conditioned for 45 minutes by 10 fish per 18 liters was run against unconditioned well water. In the controls unconditioned well water was run on both sides with the following results:

	Mean difference	*Significance*
Cond. end of experiment vs. same end of control....	.85—for cond.	.8541
Cond. end of experiment vs. 50%.....	3.05+for cond.	.3446

Although there is a slight tendency toward a positive response here the results are of no significance and probably indicate indifference.

Series XIII and XIV. These experiments were similar to Series XI and XII but the conditioned water was taken from a trough 11 ft. 6 in. long, 1 ft. 8 in. wide, and containing 6 in. of water, in which three large fishes and three turtles had lived for 10 to 12 days. This should test for any positive response to heterotypic conditioning. The results were:

	Mean difference	*Significance*
Cond. end of experiment vs. same end of control....	1.4 +for cond.	.8208
Cond. end of experiment vs. 50%.....	4.55+for cond.	.1934

The very slight tendency toward the conditioned water is insignificant.

Series XIX and XX. In all of the foregoing experiments it is possible that the conditioning of the water which favors survival in many aquatic

animals may have produced a tendency toward a positive response which had nothing to do with the aggregating behavior of the fish. Therefore in this set of experiments conditioned water of two types was used, one conditioned for one hour by 50 small catfish per 18 liters, and the other conditioned for the same length of time by an equal number of mud minnows, *Umbra limi* (Kirtland). For the controls unconditioned well water was run on both sides. These experiments were run July 23 to August 2, at a time when the catfish were aggregating closely. The animals used in these experiments had been in the laboratory for one to two weeks, and were aggregating most of the time and always when even slightly stimulated. Experimental summaries showed:

	Mean difference	*Significance*
Catfish cond. end vs. same end of control.....	1.1—for catfish	.8380
Catfish cond. end of experiment vs. 50%.....	2.7+for catfish	.5050

If there is any response to a specific conditioning it seems that it should have shown under the conditions of this experiment. The results, however, are decidedly indifferent.

Series XXI and XXII. In these experiments, run in Tank A, unconditioned well water was run against water from an aquarium 8 ft. long, 2 ft. wide, containing 10 in. of water in which about 50 catfish had lived for two months, August to October. The experimental animals were taken from another aquarium of approximately the same dimensions. They had been in the laboratory for about a month, and were still aggregating to a considerable extent. The results:

	Mean difference	*Significance*
Cond. end of experiment vs. same end of control....	4.75—for cond.	.3446
Cond. end of experiment vs. 50%.....	2.7 —for cond.	.9214

The results here are definitely indifferent, showing no positive response to the catfish conditioned water.

Series IX and X. These experiments were run to determine whether there could be demonstrated a direct response to a conditioned gradient produced by a small group of catfish in quiet water. Tank B was used with wide meshed wire partitions (covered with paraffin) and placed 3 in. from one end and 9 in. from the other. The 9 in. end was on the left in Series IX and on the right in Series X. The experimental animals were two blind fishes whose eyes had been destroyed one week before, and which had since been kept in an aquarium by themselves. They had apparently fully recovered from the operation. Well water was placed in the tank and changed after each experiment and each control. Ten normal fishes were placed in the 9 in. end for the experiments as the conditioning factor. The order of the experiment and control was varied as in the preceding series.

When the time spent in the conditioned end of the experiment was compared with the time spent in the same end of the control, there was found to

be a mean difference of 22.3% in favor of the control with a significance of .0002. In the experiment there was a mean difference of 11.25 in favor of the end away from the group with a significance of .0008. This indicates a negative response to the group. That this was due to effects from the shock of the operation is doubtful when the normal behavior of the operated animals used in aggregation experiments is considered. A more probable explanation seems to be the previous isolation from the group. This is supported by the results of the next series. A sensitivity to conditioning, if present, was not sufficient under the conditions of this experiment to call forth any positive response.

Series XVII and XVIII. This set of experiments was exactly like IX and X except that the blind fishes were kept with the group, both before and between the experiments. Here the results were indifferent:

	Mean difference	*Significance*
Group end of experiment vs. same end of control	11.5—for group	.1666
Group end of experiment vs. 50%	3.4+for group	.5960

Isolation was apparently the cause of the negative results shown in Series IX and X.

Series XV and XVI. These series were similar to IX and X, but closely woven black cloth was placed over the wire meshed partitions and normal fishes from the group were used as experimental animals. The results were as follows:

	Mean difference	*Significance*
Group end of experiment vs. same end of control	9.05—for group	.2448
Group end of experiment vs. 50%	.35+for group	.9606

Here normal fishes showed no response to a group of fish out of sight, and so gave no evidence of a positive response to a conditioned gradient.

The controls of these series with Tank B show a tendency for the fishes to stay in the 9 in. end of the tank which is significant in one of the sets, Series IX and X. The reason for this was not determined. The opposite tendency in the experiments of Series IX and X with isolated blind fishes when a group was in the 9 in. end counteracts this sufficiently to give the results significance, and is probably therefore a true indication of a modification of behavior due to isolation.

A summary of the results of the experiments with gradient tanks is given in Table I. There is no evidence that a conditioning of the water, as by some chemical substance secreted by fishes into the water, plays any important part in the aggregation of catfishes. The only positive results were obtained with very highly conditioned water by fishes which had been living in that water, and this may very well have been due to an adaptation to a high percentage of waste products, or some such factor. Since the aggregations are formed very quickly, often in the course of a few seconds, it seems improbable that a response of this type is important. These results indicate that the olfactory

organs are not concerned in this group reaction, since according to Parker (1910) they are the organs through which a distance perception of a chemical substance must occur. This does not, however, exclude the possibility of a gustatory response associated with a contact stimulus.

The negative response to the group obtained from the blinded fishes which had been isolated needs further investigation. It may be that isolation has some effect upon the reaction to vibrations which seems to be the stimulus, if any, which was effective.

TABLE I

GRADIENT TANK EXPERIMENTS

FLOWING WATER IN TANK A

Series	*Conditions*	*Mean difference in %*	*Significance*
I, II	Colored well water vs. long conditioned water	1.4	.8514
		.....29.6	.0006
	Colored well water vs. clear well water	28.2	.000,000,1—
III, IV	Clear well water vs. long conditioned water	11.65	.005
		.....22.15	.0004
	Clear well water vs. clear well water	10.5	.0494
XI, XII	Well water vs. water conditioned for 45' by 10 fish per 18 liters	3.05	.3446
		..... .85	.8514
	Well water vs. well water	3.9	.3848
XIII, XIV	Well water vs. water conditioned heterotypically for 10 days	4.55	.1934
		..... 1.4	.8208
	Well water vs. well water	3.15	.4688
XIX, XX	Minnow cond. water vs. catfish cond. water	2.7	.5050
		..... 1.1	.8380
	Well water vs. well water	3.8	.3398
XXI, XXII	Well water vs. water cond. by catfish for two months	4.75	.3446
		7	.9214
	Well water vs. well water	4.05	.3956

QUIET WATER IN TANK B

Series	*Animals*	*Conditions*	*Mean difference in %*	*Significance*
IX, X	Isolated blind fish (wide meshed partitions)	← 0 fish vs. 10 fishes	11.25	.0008
			.....22.3	.0002
		→ 0 fish vs. 0 fish	11.05	.0084
XVII, XVIII	Blinded fish from group (wide meshed partitions)	- - - → 0 fish vs. 10 fishes	3.4	.5960
			.....11.5	.1666
		- - - → 0 fish vs. 0 fish	14.9	.0228
XV, XVI	Normal fish (black cloth partitions)	- - - → 0 fish vs. 10 fishes	.35	.9606
			..... 9.05	.2448
		- - - → 0 fish vs. 0 fish	9.4	.0967

* The arrows designate the end favored to the extent of the mean difference indicated. Where this is significant the arrows are represented by solid lines, where it is not significant by dotted lines.

TABLE II

Stages of aggregations of ten blinded fishes after given intervals of time.

	Exp. 1 — in light		*Exp. 2 — in light*		*Exp. 3 — in dark*	
	Interval	*Stage*	*Interval*	*Stage*	*Interval*	*Stage*
1	2'50"	5	1'	2	1'46"	5
2	2'30"	3	1'	4	1'21"	4
3	2'20"	4	1'	4	1'25"	3
4	2'10"	4	1'30"	3	1'15"	5
5	1'30"	3	1'	4	1'11"	4
6	1'05"	4	1'	4	0'58"	3
7	1'35"	4	1'	4	1'35"	3
8	1'03"	5	1'	4	1'14"	3
9	1'30"	4	1'	4	1'16"	5
10	1'02"	4	1'	5	1'50"	3

AGGREGATION EXPERIMENTS

In this part of the investigation groups of fish were used and their aggregating behavior was studied directly. The group was placed in a white porcelain tub, 3 ft. 3 in. by 2 ft. 1 in., which contained 3 to 4 in. of water. The experiments were carried on in a dark room. A 40 watt light placed over the center of the tub gave a fairly even lighting, and could be switched on and off at will. For convenience the following five stages in the degree of aggregation were decided upon, and the results recorded in terms of these stages:

Stage 1. Close aggregation, fishes in one or two compact groups.
Stage 2. Loose aggregation.
Stage 3. Slight grouping, one half of individuals near another fish.
Stage 4. Mostly scattered, less than one half of individuals near another fish.
Stage 5. Completely scattered.

In a tub of this size with ten fishes, there is a distinct difference between the extremes of this series. Stages 3 or 4 might possibly appear by chance in a few cases when actually there was no aggregation, but never Stages 1 or 2.

The operations on the different sense organs were in most cases performed according to the technique worked out by Parker (1917). The olfactory nerves were cut by an incision just posterior to the olfactory pits. The auditory nerves were cut by an incision between the ear and the brain. The lateral line organs in the head were destroyed with an electric needle, while the lateral nerves were cut by an incision at the anterior end of the lateral line canal. The skin was anaesthetized by treatment with a 10% solution of magnesium sulphate in most cases for two minutes. This was a more dilute solution and was used a shorter period of time than that employed by Parker, but the fishes used were small and a stronger solution or a longer treatment was either fatal or prevented all activity for an hour or more. In the experiments on older fishes treatment for five minutes was necessary to render them insensitive to touch. In blinding, the eyeball was destroyed with a hot needle in the first few cases. Sometimes this operation was not complete and a part of the eye was apparently reconstituted after a few days. Therefore in most of the animals used in this type of work the eyeball was removed completely. The fishes recovered rapidly with no fatalities. Blinders, as used by Parker, were not feasible on these small fishes. The reactions of the blinded fishes were in all cases such as was expected from the behavior of the normal fishes in the dark. They were active almost continuously and remained isolated from one another. For all of the operations the fish were anaesthetized in a solution of 10% ether in water until they were entirely inactive. This took from two to five minutes depending upon the size and condition of the fish.

Vision

The first three experiments were performed to test the statement made by Eddy (1925), that blinded catfishes do not aggregate. In Exp. 1 ten fishes which had been blinded by a hot needle two days before were placed in the tub and the stage of aggregation noted at given intervals. These trials were made in the light. In Exp. 2 and 3 ten fishes were used which had been blinded by the removal of the entire eyeball two days before. Exp. 2 was carried on in the light and Exp. 3 in the dark. In the last case the stage of aggregation was observed at the instant the tub was illuminated.

The results are shown in Table II. In no case was Stage 1 observed and Stage 2 in only one case, and that at the beginning of Exp. 2 before there had been sufficient time for the fish to separate to any extent. There is no indication of any greater aggregating behavior in the light than in the dark. This is in marked contrast to the aggregations of normal fish as will be shown later.

It seemed desirable to check these results by determining whether under any conditions normal fishes would aggregate in the dark. Preliminary observations were made on 100 small catfishes which had been brought into the laboratory about a week before. These were placed in the tub with a 40 watt Mazda light over the center. The behavior was noted as follows:

Light—Closely aggregated.
Dark for 1′ 43″—Loosely scattered.
Light—Aggregated closely in 2″.
Dark, followed by dim light from far end of room—Loose aggregation, very active.
Scattered mechanically—Equally distributed throughout the aquarium, active.
Light—"Streamed" together, 5 groups, then 2, then 1 in 30″.
Dim light for 3′—Even scattering over the aquarium.
Light—Aggregated in 3″.

A series of experiments was then run on groups of ten fishes to test more fully the relation of the stage of aggregation to the presence of light.

In Exp. 4 ten normal fishes were placed in the tub in the light, and the stage of aggregation recorded at ten second intervals for 20 trials. In all the observations the fishes were in a close aggregation, in Stage 1.

In Exp. 5 ten normal fishes were placed in the tub, left for intervals in the dark, and observed as the light was flashed on. The results in Table III show no close aggregation, and only one case of Stage 2 at the beginning. Observations after shorter periods in the dark showed that the amount of scattering varied directly with the time since illumination. In general the groups were in Stage 2 after 15″, in Stage 3 after 30″, and in Stage 3 or 4 after 45″.

When catfishes are scattered in an aquarium in the laboratory, as frequently occurs when the room has been quiet for some time, they will always aggregate quickly if the aquarium is struck once or twice. To determine whether the fishes would aggregate in the dark if disturbed, in Exp. 6 a series of ten trials was made with two parts to each. In the first part the stage of aggregation was observed after a period in the dark as in Exp. 5. In the second part a blow was struck on the tub three to five seconds before the light was turned on and the stage recorded. The results given in Table 3 show no aggregation of any significance in either part.

There was the possibility in Exp. 6 that there had not been time for aggregation in the dark which, if possible, might take longer than in the light. To check this in Exp. 7 twenty trials were run with observations made after several blows extending over a longer time than in the preceding experiment. The trials were run at four different times, five trials at each. There is no evidence of aggregation from these experiments.

TABLE III

Stages of aggregation of ten normal fishes in the dark with and without disturbances from blows.

	Exp. 5		*Exp. 6(a) No blow*		*(b) 3"-5" after blow*	
	Interval	*Stage*	*Interval*	*Stage*	*Interval*	*Stage*
1	0'54"	2	1'20"	5	1'10"	5
2	1'44"	5	1'30"	5	1'16"	5
3	1'40"	5	1'10"	5	1'30"	5
4	2'08"	4	1'27"	5	1'12"	5
5	1'52"	5	1'03"	3	2'05"	5
6	2'14"	5	1'07"	5	2'15"	5
7	1'03"	5	1'10"	5	1'07"	3
8	0'54"	3	1'15"	5	0'35"	3
9	1'40"	3	5'05"	3	0'40"	5
10	0'59"	3	0'55"	5	0'50"	5

	Exp. 7						
	(a) No blow		*(b) 3"-5" after blow*		*(c) Longer interval with blows*		
	Interval	*Stage*	*Interval*	*Stage*	*Interval*	*Blows*	*Stage*
1	1'39"	5	1'38"	5	1'45"	0'40"	3
2	1'29"	4	1'40"	5	2'12"	37"	5
3	1'52"	5	2'10"	4	0'52"	23"	5
4	1'15"	5	0'58"	5	1'13"	31"	5
5	1'54"	5	2'30"	5	1'34"	17"	5
6	1'10"	4	1'55"	5	1'51"	23"	5
7	1'57"	5	1'48"	5	2'14"	26"	4
8	1'58"	5	0'51"	3	1'30"	17"	5
9	1'13"	5	0'59"	5	1'07"	17"	5
10	1'06"	5	1'13"	5	1'22"	25"	5
11			1'27"	5	1'22"	20"	5
12			1'10"	3	1'40"	21"	5
13			2'05"	5	1'14"	33"	5
14			1'25"	5	1'33"	15"	5
15			1'26"	5	1'19"	15"	5
16			1'50"	4	1'35"	29"	5
17			1'40"	5	1'20"	20"	5
18			1'20"	5	1'53"	30"	5
19			1'15"	4	2'25"	24"	5
20			1'16"	5	2'08"	29"	5

Observations were also made at hourly intervals throughout the night on the behavior of catfishes in two aquaria in the laboratory. Aquarium A, measuring 8 ft. long, 2 ft. wide, and containing 10 in. of water, faced the window and had the sides of the left end covered with black paper. Aquarium B, measuring 4 ft. long, 2 ft. wide, and containing 10 in. of water, was located with the left opaque end toward the window and so in the shadow. The results from these observations are given in Table IV. They show no aggregation during the darker part of the night, a gradual increase in the closeness of aggregation as it began to grow lighter, with aggregations similar to those during the day at dawn. There was some illumination entering the window of the laboratory, so that the fishes were not in absolute darkness. Since, however, these fishes were collected from a ditch along a main highway frequently well lighted by passing automobile lights, the illumination was probably not much greater than occurred in their natural habitat. No

case of an aggregation approaching those typical of the daytime was observed in either aquarium during the night.

A group of catfishes give reactions to large moving objects which correspond closely to those reported by Garrey (1905) for a school of sticklebacks. They move in the opposite direction from the object and thus escape pursuit. To a small moving object about their own size they give quite a different reaction, and swim along parallel to it or directly behind it. This reaction is induced when a black paraffin model of a catfish is drawn through the water, or even when a piece of rubber of the same size is substituted.

All of the results on vision indicate that it is a limiting factor in the formation of aggregations, and that without it aggregations break up in the course of a few minutes. According to these results the catfishes must be scattered in very muddy water and at night, and come together where vision is possible. The reformation of a catfish aggregation after scattering in the ponds where they are found suggests that this is probably the case (p. 1).

Equilibration and Hearing

An attempt was made to determine whether the ear played any part in the aggregating behavior. The eighth nerves were cut by dorsal incisions on 12 fishes of which 10 survived for two days or more. The completeness of the operation was shown by the loss of equilibrium in all cases. This, however, interfered with any possible tendency to aggregate since the fishes could not direct their movements. They turned in spirals while swimming, and rested unless disturbed. No other attempt was made to ascertain whether hearing is concerned, but it seems extremely doubtful that it is. The only sound the young catfish were ever heard to make was a slight cracking noise when they opened their jaws widely. This occurred only when they were handled as in the anaesthetizing experiments, and was never observed in the aquarium.

Olfactory Sense

Two groups of fishes were used in these experiments. Group A consisted of eight fishes which survived from a group of ten which were operated upon July 16. Autopsies performed July 19 at the conclusion of the experiment showed seven of the eight operations to be complete. In three cases the olfactory nerves had been cut, in four cases the olfactory lobes were severed and in one instance the cerebral hemispheres were injured. Group B consisted of ten fishes operated upon October 10, all of which survived. On November 18 the operations were repeated upon five of these to offset a possible regeneration of the nerves, and at the same time the eyeballs were removed. Autopsies performed December 3 upon the four individuals still surviving showed that in these cases the operations had been complete.

In Table V are given the stages of aggregation shown by these two groups of fishes at intervals. It will be seen that the aggregating behavior was not at all affected by this operation.

A single experiment with normal fishes was performed in connection with the distance perception of chemicals from other fishes. Eight individuals were placed in a small aquarium, 10′ by 8′ by 8′, in which were suspended two cheese cloth bags, one containing a piece of glass rod, and the other two pithed catfishes. In 25′ there was no response to either bag, but with liver in place of the pithed fishes there were 58 nibbles at the bait in 30′ and four at the glass rod. In this last experiment the bags were interchanged at the end of 15′.

Another experiment was run with three live fishes tied in the cheese cloth bag. This was of 30′ duration with the bags interchanged at the end of 15′. Here there were five nibbles at the control and two at the bag which contained the fishes. This gave no indication of a perception of other fishes through the olfactory organs.

The results of these experiments agree with those from the gradient tanks in giving no evidence for any distance perception of a chemical as a factor in the aggregation of catfishes.

In some of the earlier experiments on blinded fishes it was observed that occasionally, in cases where there could be no question of the completeness of the operation, a blinded fish would turn toward another fish swimming past and sometimes follow it for one or two turns. This suggested that, whereas a fish can never keep close to another fish except by means of vision, it may be sensitive to the presence of another fish through other sense organs than the eyes. To determine whether this is so a series of experiments was run on blinded fishes, with and without other sense organs. A group of fishes, usually five, was placed in the porcelain tub. The blinded fishes remained scattered, ordinarily swimming at random around the tank. Each time two fishes passed within two inches of one another going in the opposite direction the reactions of both were noted. If one of the fishes turned toward the other or altered its direction as if to turn back and follow the other, it was considered a positive reaction. If neither gave any reaction, it was considered indifferent. If one fish turned away from the other without turning back it was considered a negative reaction. In any case where the stimulus may have been something besides the other fish (*e. g.* the side of the tub) the reaction was not recorded. These criteria were based on the similar but better directed reactions of normal fishes to each other. Since no positive reactions of blinded fishes were observed when the fishes passed at more than two inches from one another, this was taken as the maximum distance to be considered. In some of the experiments the reactions were also recorded when two fishes actually touched each other in passing. In some instances these showed a larger percentage of positive reactions, but there were usually fewer of them, and in many instances there were approximately the same proportion of types of responses as when there was no actual contact between the fishes. The experiments ran for 15′ in all cases.

The results are given in Table VI. Exps. 1 and 3 give the percentages with normal animals. In Exp. 2, the first one with blinded fishes, there was no indication of a positive response. The normal fishes, however, were not at this time showing typical aggregating behavior. Only two of the five blinded animals were active for most of the time, and almost all the positive reactions were given by these. The other three were resting, something which never occurs normally under the conditions of the experiment. This experiment was performed during very hot weather when the fishes were dying in large numbers. There were only 7 per cent positive reactions and these may well have been due to chance. In all the other experiments (4, 5, 6, 9, and 14) blinded fishes gave about 50 per cent of positive reactions. The results of Exp. 2 are included only to indicate that fishes in a poor condition do not give reactions. None of the other treatments given to catfishes inhibited locomotion, with the possible exception of treatment with magnesium sulphate.

By comparing the results of Exps. 7 and 8, in which the olfactory nerves were cut, with those in which the olfactory nerves were intact, it will be seen that the cutting of these nerves has no effect upon this response of the blinded fishes. After cutting the olfactory nerves there was 47.5 per cent of positive responses as compared with 47.8 per cent in fish which were blinded only. Positive responses similar to those produced by another fish were frequently observed if a paraffin model with a loose movable tail was drawn past a fish. In this case there was little or no possibility of a specific chemical stimulus.

TABLE IV

Observations at hourly intervals during the night upon two aquaria of young catfishes.

Time	*Large aquarium A*	*Small aquarium B*
11:00 P.M. (After several hours in light)	Loose aggregation behind dark paper	Active, more or less scattered
11:30 (After 30′ in dark)	Actively swimming and scattered, more in lighter end of aquarium	Same as A
12:00	Active, scattered, few more in lighter end	
12:05	(After 5′ in light with frequent blows) 2 small close aggregations in lighter part, large loose aggregation in darker part	(After 5′ in light) One large fairly loose aggregation in the light end
1:00	Active, scattered, many more near surface at lighter end	Same as A (After 1′ with jarring) One loose aggregation
1:05	(After 5′ in light) Two very loose aggregations, many scattered	
2:00	Scattered, but more in lighter end	Same as A

3:00	Same	Same
4:00	Evenly distributed in light third of aquarium	Loosely collected at light end
4:30	Same	Same
5:00	Loose aggregation, few scattered	Same as A
5:15 (Sunrise)	Aggregated	Aggregated

Perception of Vibrations

Since a chemical factor is apparently not concerned in the positive reactions of blinded fishes to one another, it seems possible that they may be due to the vibrations set up in the water by the tails of the fishes as they swim past one another. This is suggested by the fact that there seem to be more positive reactions when the fishes swim rapidly and vigorously than when they swim in a more leisurely manner. The tails vibrate about four to six times per second in fast swimming, and fairly regularly. As shown by Parker (1917) vibrations in water are received by Ameiurus through three sets of sense organs, the ears, the lateral line organs, and the skin. The most rapid vibrations, received by the ear, are probably not concerned with aggregation responses. Experiments were performed to determine whether the lateral line organs or the skin were concerned.

When the skin was anaesthetized with magnesium sulphate the percentage of positive reactions was very small, as shown in Exps. 10, 11, 12, and 19 (Table VI). This treatment tended to make the fishes more sluggish, but in both 11 and 12 the number of possible reactions was fairly large. As a partial control an experiment was performed in which fishes were given the same treatment with water. These fishes showed a much higher percentage of positive reactions, 35%, as shown by Exp. 13. Close aggregations were formed when fishes with normal vision were treated with magnesium sulphate (Table V), although these fishes were less active than untreated fishes and rested in groups a part of the time.

Fishes with lateral line organs destroyed aggregated in a normal way as shown in Table V. When blinded and tested for individual reactions, they gave a percentage of positive responses comparable to those given by blinded fishes with lateral line organs intact (Exps. 15 and 17). Apparently positive reactions were not dependent upon the lateral line organs. When these same fishes were anaesthetized with magnesium sulphate, the percentage of positive reactions was insignificant (7 and 8 per cent). These results indicate that the skin is the receptor of the stimulus responsible for aggregations. The average percentage of positive reactions for the experiments of each type and the number of possible responses for each are given in Table VII. Blinded fishes gave approximately 50 per cent of positive reactions, except when the skin was anaesthetized. Whether the reaction

to vibrations received through the skin is of any fundamental importance in this connection is questionable. It may be only a conditional automatism which has been developed in connection with the aggregating behavior. This point needs further investigation.

Tactile Reactions

Catfish, as shown by Schiche (1921), are strongly thigmotactic, always resting with as many points of the body as possible in contact with the substratum. This response also plays a very important part in the aggregating behavior of these animals, reinforced as it seems to be by a response to gustatory stimulation. When two fishes come to rest in an aquarium they are usually touching or very nearly so. When normal fishes are placed in a tub of water with black paraffin models, they will frequently approach a model, rub the barblets over it, and then turn away. This occurs repeatedly, whereas if they approach a resting catfish in the same way, they push against it several times and usually come to rest touching it or nearby. Such behavior was observed toward a sick fish resting on its side, and to a freshly killed fish held in place by a twig. In the latter case the living fish twice pushed against the dead one until it was dislodged and floated to the top. When mud minnows were placed with catfishes the latter approached a resting minnow, drew the barblets over it, pushed against it several times, and frequently came to rest in contact with the minnow if the latter remained quiet. Usually the minnow swam off. Catfishes follow mud minnows and goldfishes in the aquarium, but the minnows avoid the catfishes with a darting movement, and thus escape, while the goldfishes swim more rapidly, or dodge out of the way. When the barblets were removed the catfish still aggregated quite normally. There are indications here of a tactile-gustatory discrimination of some kind, but not such as enables a catfish to distinguish members of its own species.

TABLE V

Stages of aggregation of fishes with eyes intact and other sense organs inactivated.

	Olfactory nerves cut A. 8 fishes	B. 10 fishes	*Lat. line organs destroyed* 5 fishes		*Skin anaesthetized* 8 fishes			
	Int.	*Stage*	*Int.*	*Stage*	*Int.*	*Stage*	*Int.*	*Stage*
1	1′30″	1	1′	1	1′	2	1′	2
2	1′	1	1′	1	1′35″	1	1′	1
3	1′	1	1′	1	2′	1	1′	1
4	1′07″	1	1′	1	1′30″	1	1′	1
5	1′20″	1	1′	1	1′30″	1	1′	1
6	1′03″	1	1′	1	1′	1	1′	1
7	1′	1	1′	1	0′30″	1	1′	1
8	1′20″	1	1′	1	1′	1	1′	1
9	1′	2	1′	1	1′	1	1′	1
10	1′	1	1′	1	1′	1	1′	1

TABLE VI

Individual reactions of fishes to others of group.

Condition	*No. of fishes*	*Non-touch reactions* +	—	0	*Tot.*	%+	*Touch reactions* +	—	0
1. Normal.....	5	147	2	13	162	91%	3	1	1
2. Blinded (2)*.....	5	5	8	58	71	7%	2	7	2
3. Normal.....	5	All aggregated whole period							
4. Blinded (2).....	5(2 cm.)	14	1	20	35	40%	7	2	3
5. Blinded.....	5(3.5 cm.)	28	1	25	54	52%	9	6	4
6. Blinded (11).....	5	26	0	21	47	55%	6	1	4
7. Blinded (11)..... Olf.N. cut (3)	5	25	1	21	47	53%	12	2	0
8. Blinded (2)..... Olf.N. cut (2)	5	5	0	7	12	42%			
9. Blinded (3).....	5	13	3	12	28	46%	5	0	3
10. Blinded (3)..... Tr. MgSO4	5	0	0	3	3	0%	0	0	0
11. Blinded (12)..... Tr. MgSO4	5	7(4)	4	31	42	17%	5	7	5
12. Blinded (29)..... Tr. MgSO4	3	2	3	18	23	9%	3	2	2
13. Blinded (29)..... Tr. H_2O	3	7	2	11	20	35%	3	1	2
14. Blinded (31).....	5	25	0	29	54	46%			
15. Blinded (1)..... Lat. L. O. dest. (1)	5	27	0	28	55	49%			
16. Blinded (1)..... L.L.O. dest. (1) Tr. MgSO4	5	4	1	56	61	7%			
17. Blinded (57)..... L.L.O. dest. (2)	5	26	0	27	53	49%			
18. Blinded (60)..... L.L.O. dest. (5) Tr. MgSO4	5	2?	0	22	24	8%			
19. Blinded (61)..... Tr. MgSO4-5′	5	1?	0	12	13	8%			

TABLE VII

Percentage of positive reactions shown by passing fish
Summary of experiments

Normal % Pos. Tot.	*Blinded* % Pos. Tot.	*Olf. N. cut* % Pos. Tot.	*Lat.L.dest.* % Pos. Tot.	*Tr. MgSO4* % Pos. Tot.	*Lat.L.dest. &c tr. MgSO4* % Pos. Tot.
91% 162	(7% 71)	53% 47	49% 55	0% 3	7% 61
100%	40% 35	42% 12	49% 53	17% 42	8% 24
	52% 54			9% 23	
	55% 47			8% 13	
	46% 28				
	46% 54				
Aver. %					
95.5%	47.8%	47.5%	49%	8.5%	7.5%
Totals	176	59	108	81	85

*Numbers in parentheses indicate days which elapsed between operation and experiment.

DISCUSSION

Aggregations are of widespread occurrence throughout the different phyla of the animal kingdom, and are found in varying degrees of complexity; from the chance assemblages of animals resulting from some external environmental factor or group of factors, to the highly organized groups among social insects and man, based almost entirely upon the reactions of the individuals to one another, and containing undoubtedly what is usually known as a social element. When, however, an attempt is made to classify animal groupings and to draw a line of distinction between chance aggregations and those which are clearly social, many difficulties are encountered. Attempts have been made by several authors, but none of these are entirely satisfactory. They serve largely to emphasize the fact that there is no clear distinction between different levels of complexity in animal aggregations, but that a continuous series may be traced from the simplest to the most complex. This fact suggests the possibility that all animal aggregations may have some elements in common, and that analysis of some of the simpler cases may throw light on the more highly organized types of integration. The study of animal groups is included in what has usually been called general sociology.

To understand social behavior in animals, their integrations, and the nature of the satisfaction of the social drive or "social appetite" some sort of analysis is necessary. One logical approach to this problem is through a study of the sense organs. They must serve as the gateways by which all sensations from the external world reach the central nervous system, and therefore all stimuli from other individuals must pass through them. Analyses to determine the sense organs concerned in integrations and in the satisfaction of the social appetites should furnish a firmer basis for the classification of animal societies and knowledge of their evolution than a purely descriptive foundation. Aggregations of young catfishes are peculiarly interesting for studies of this kind. They belong among those groups of animals which have a distinctly social aspect as contrasted with chance aggregations of animals due to the force of external factors; and the degree of integration is high, as any one will appreciate who has seen one of the closely packed groups. But the organization is extremely simple, with nothing approaching in the slightest degree a morphological division of labor, and with the individuals of the mass constantly shifting in what is apparently a purely haphazard manner. Thus it seems possible here, if anywhere, to find clues to the fundamental reactions concerned in this type of social behavior. In addition catfishes are excellent experimental material. They survive operations very well, and considerable work has been done on their individual reactions.

The first sense organs which were studied by the writer in connection with the group behavior in young catfishes were the olfactory organs, a pair of pits located in the anterior end of the snout, and connected with the olfac-

tory lobes of the brain by the olfactory nerves. These were readily severed in the operated animals by a dorsal incision. Parker (1910) has shown that these organs function as distance receptors for chemical stimuli. The chemical receptors in the skin, the taste buds, are, on the other hand, sensitive to chemical stimuli when in more or less direct contact with them (Herrick, 1902; and Parker, 1910). Conditioning of the water by animals of the same or of other species has been found to have survival value for many animals (Drzewina and Bohn, 1928; Allee, 1929a, and citations). Various theories have been advanced to account for this fact, among them that of the secretion of some auto-protective substance into the water. It seemed possible that, if such group protection held true for young catfishes, individuals might collect into groups on account of the perception of some substance. At least it was possible that some specific chemical might serve as an integrating factor in the formation of the aggregations, even if no protective value were inherent in it. To test this possibility the gradient tank experiments were performed with water conditioned homotypically and heterotypically for varying lengths of time. Since catfish aggregations may form in a few minutes no long conditioning could be expected to effect them. In only one case did the results of the writer's experiments give support to the idea that aggregations were due to chemical substances. This was when highly heterotypically conditioned water was used. In this there must have been an overabundance of waste products, and the experimental animals used had been living in this water and had become adapted to it. This amount of conditioning could not effect ordinary aggregations.

It was yet possible that the aggregations might depend upon a specific chemical which would lose its effectiveness in the water and stimulate for only a short time after being given off. The experiments with the fish in the cheese cloth bags were performed to test this. The normal animals gave the expected number of positive reactions to liver suspended in the bags, such reactions as were shown by Parker (1910) to be due to a stimulation of the olfactory organs, but gave no more positive responses to bags containing pithed fishes than to those with a glass rod. It should be noted here that normal fishes showed positive reactions toward a pithed fish when actual contact occurred, and were able to distinguish it from a paraffin model.

The third piece of evidence in regard to the olfactory organs is from the experiments with operated animals. Fishes with eyesight intact, but with the olfactory nerves cut, aggregated in a manner in no way distinguishable from that of normal fishes. When blinded these fishes gave a percentage (47.5) of positive reactions entirely comparable to that (47.8) shown by blinded fishes with the olfactory nerves intact. The cutting of the olfactory nerves had no discernible effect upon the aggregating reactions.

From these three lines of evidence there is no indication that the olfactory sense organs play any part in social behavior of the young catfishes, or

that a specific chemical that can be perceived at a distance acts as an integrating factor, as is the case in some of the social insects. This is true in spite of the fact that the olfactory organs themselves are fairly sensitive and of primary importance in the feeding of catfishes (Parker, 1910).

Hearing has not been investigated with any thoroughness. Catfishes do have a fairly well developed sense of hearing as has been shown by von Frisch (1923), who has trained a catfish (*Ameiurus nebulosus*) to feed at the sound of a whistle. But since there is no evidence that catfishes make any sound while aggregating, it is unlikely that hearing is important in integration.

Sense organs which are of great importance in the integration of catfish aggregations are the eyes. This was shown by Eddy (1925). The results of this investigation have strongly corroborated his findings. Blinded fishes never aggregated, and in addition it was shown that catfishes do not aggregate in the dark, and that aggregations are broken up in about a minute after light is removed. Aggregations do not form in the dark even when the fishes are disturbed by noises and jarring, stimuli which almost always produce rapid aggregations in the light. All observations indicated that the fishes studied were in motion most of the time in the dark as are the aggregating fish in a lighted aquarium, but there was no schooling. Older fishes in the light usually rest in as shaded a region as possible, and when disturbed dart rapidly about as if highly stimulated. With small fishes the aggregating responses overcome the negative reaction to light when more than one fish is present. It can be concluded from these observations that the eyes are extremely important for integration and that without them the aggregations cannot form. They are the means for "distance integration" by which the fishes are brought into proximity with one another.

The manner in which the eyes function in bringing about such integration is as yet largely a matter for conjecture. Lyon (1904, 1905) has shown that stream fishes keep their location in the rapidly flowing current by maintaining the retinal image of the objects on the bank in a fixed position. Here the fish move so as to keep the whole background in the line of vision. Brown and Hatch (1929) in an analysis of the reactions of gyrinid beetles, have shown that they give the "fright" reaction to a stimulus which increases the complexity of the visual field, as when a white spot is introduced upon a black background or vice-versa. The aggregations of these beetles also disperse in the dark. A normal young catfish invariably turns and follows another fish swimming in the direction opposite its own, or even in a direction at a slight angle to that of its course. If two fishes are put into a tub at opposite ends, they will remain separate until by chance they come within two or three feet of each other; then one swims directly toward the other and follows it. This same response is given if a rough black paraffin model of a fish or any small object of about the same size is drawn past an individual. In this case the fish ceases to follow the model after a few feet. It seems as if the reaction in

a catfish were a combination of the type described by Lyon and that described by Brown and Hatch. The stimulus is given by the appearance in the field of vision of the configuration of a small moving object. The fish responds by attempting to keep this object in a fixed position on its retina. Uvarov (1928) describes a similar reaction for grasshoppers:—"A sudden movement of an object in the field of vision of a hopper results almost invariably in a reflex jump." These jumps stimulated by moving companions and occurring in the same direction as that of the movement of the stimulating individual, Uvarov believes, cause mass movements of the bands of nymphs. Whether the visual reactions to a small moving object on the part of the young catfish is an inherited reflex or a conditional response has not yet been determined.

To large moving objects, a moving hand for instance, appearing in the field of vision, the fish responds by turning in the opposite direction. This type of behavior is similar to that described by Garrey (1905) for sticklebacks, and explained by the fact that the motion of a near object causes an apparent motion of the background in the opposite direction, and stimulates the fish to keep the retinal image of this background in a constant position. There seems to be a difference in the response of catfish to moving objects of different sizes. Just where the line of division comes has not been investigated.

Since Parker (1910) had shown that the ears, the lateral line organs, and the skin are all receptors of vibrations, but that there are differences in the frequencies of vibrations to which they are sensitive, these three sense organs were studied together in an effort to see whether vibrations play any role in integration in addition to that played by vision. When the fishes were blinded a large percentage of positive reactions was still given when two fish passed within two inches of each other. The stimulus which seems most probably the one working in this case is from the vibrations set up by the tails of the swimming fishes which vibrate approximately six times per second, a frequency to which Parker found the skin responsive. The results with animals on which certain senses had been eliminated by operation indicated that the skin was the organ concerned. The destruction of the lateral line organs had no effect upon the percentage of reactions while the anaesthetizing of the skin reduced the number to such a small percentage that the responses may well have been only apparent, as it is entirely possible that a fish may occasionally turn toward another by chance when no stimulus is received. Responses to vibrations are not important as integrating factors, and are not sufficient to produce aggregations in the absence of vision, as fishes soon separate from each other when blinded. The orientation of fishes through the reception of such stimuli is not possible with any degree of accuracy for a blinded fish rarely makes more than one correct turn toward a fish.

Uvarov cites another interesting case as reported by Vayssière (1921), where movements resulting in animal groupings have been described for the grasshopper, *Dociostaurus maroccanus*. An adult of this species may take off when another individual flies above it, although it will not respond to a stone thrown overhead. A sudden approach to such an insect also results in a jump. This reaction is apparently not due to sight for Lepiney (1928) found that blinded locusts responded to such an approach but that normal individuals put behind a glass did not. In both of these cases vibrations could stimulate a positive response. Whether such stimuli alone can hold together a band of locusts has apparently not been determined.

The skin, in addition to its function of receiving the stimuli of vibrations, is the means by which the strongly positive thigmotropic appetite of these animals is satisfied. That this tropism is an extremely important one in the individual reactions of these fishes has been shown clearly by Schiche (1921), who found that it was surpassed only by negative chemotropism to harmful substances, and equalled in strength the negative phototropism of these fishes. But thigmotropism is not the only response concerned in the aggregations of catfishes. Fishes in older stages which are not actively aggregating nearly always rest in contact with one another, with the tips of the barblets, the ends of the fins or the tail resting against a companion. In two small aquaria, 10 in. by 8 in. by 8 in., each containing three catfishes which were no longer aggregating, observations were made daily for six days, and the position of the fishes was recorded. These aquaria were supplied with sandy bottoms and several plants so that there was plenty of opportunity to satisfy thigmotactic responses without contact with other fishes. Of the twelve observations made, however, ten showed the three fishes in contact or nearly so, while in the other two cases two were in contact and one isolated. The cases where the fishes were not quite in contact may be explained by the fact observed many times that often two fishes come to rest in contact and then drift slightly apart without moving. In six observations made on one pair of fishes, three showed the fishes in contact and three separated. These observations indicate that the aggregating behavior of the active young has its counterpart in the quieter behavior of older fishes, and that here it is quite clearly a contact response with some discrimination, for the fishes rest more frequently in contact with each other than with other objects.

In normal aggregating there is a constant pushing throughout the cluster. Fishes on the outside push in toward the center, pushing out others which immediately push back into the group. This pushing was not as evident in the highly stimulated animals observed in the experimental tub under an overhead light as in a quiet aquarium, but when the skin was anaesthetized before the fishes were placed under observation the pushing was more marked. From an anthropomorphic viewpoint it looked decidedly as if the fishes were trying to get a stimulus which was not produced as easily as usual. This

response to contact does not seem to be due to touch alone. A normal catfish will approach a freshly killed one and push against it persistently, even though there is no possibility of reciprocal behavior on the part of the dead animal. But if a paraffin model of a fish is supplied, although the approach of the active fish is the same, it turns away after dragging the barblets over the model once. The barblets are covered with taste buds, as Herrick (1902) has demonstrated. The tactile-gustatory response is not species specific for a catfish will push against a resting goldfish or mud minnow as readily as against another catfish. The goldfish or mud minnow, however, responds by swimming away and no aggregation is formed.

Contact then is extremely important in these catfish aggregations. The observations so far seem to indicate that it serves both as a means of integration, and still more fundamentally as the principal basis of the social appetite. The satisfaction of this appetite in these distinctly social animals is apparently obtained chiefly through thigmotactic and gustatory stimuli. The reaction given to vibrations by blinded fishes may be only a thigmotactic response also; at least the stimulus for it is received through the skin. The question as to whether such a contact response is the all-important one as well as that of the origin of the visual reaction must be answered by a determination of the part which conditioning plays in this whole behavior pattern. Fishes clearly have the ability to learn as has been shown by Churchill (1916), Hineline (1927), and others. The young of Ameiurus are kept in the nest by one or both of the parents where they are in constant contact with one another for several days, and where there is an opportunity for the development of conditioned automatisms. A slight amount of evidence is at hand indicating that the responses of blinded fishes to vibrations from other fishes is lost during isolation. This is indicated by Series IX and X or the gradient tank experiments where blinded fishes after isolation responded negatively to the end containing the group of fish. But this whole subject needs further investigation before any definite conclusions can be drawn. The writer's results should be interesting in view of the fact that catfish aggregations present an example of a very simple type of vertebrate society. The reciprocal pushing of individuals apparently gives a much greater thigmotactic stimulus and may produce conditioned automatisms which result in the tendency toward group formation.

That physical contact is an important factor in many aggregations has been brought out by Allee (1927) in his survey of the field of animal aggregations. This has been shown by his investigations on land isopods (1926) and the ophiuroid, *Ophioderma brevispina* (1927). In both of these animals aggregations occur under adverse conditions, and, at least in the case of the isopods, are in part due to responses to environmental factors, but as far as reactions to other individuals of the species are concerned the aggregations of both are responses to contact. In the case of the isopods trial and

error movements are made by individuals until contact occurs, after which the animals remain together in an aggregation. Bunching in such animals has survival value because it markedly decreases the rate of water evaporation and lowers the respiratory rate. Ophioderma will aggregate with glass rods in a typical fashion when marine eel grass or other more favorable elements of the substratum are lacking. Although the reciprocal element is not as important among ophiuroids and isopods as in catfishes, for with them aggregations occur in the presence of plants, yet there are evidences that it plays some part, as the effect of glass rods on the respiratory rate of Ophioderma does not equal the effect of the presence of other ophiuroids. A similar case is described by Krizenecky (1923) for the annelid, *Enchytraeis humicultor,* where temporary contact aggregations are produced by vigorous stirring.

The pioneer work in an analytical study of the beginnings of social behavior in animals was that of Szymanski (1913). This investigator distinguishes two types of social behavior reactions: (1) primary reactions which are defined as the sum total of individual reactions apart from any social influence, and (2) secondary reactions which have arisen as a result of long living together of many individuals. To distinguish these he first found all the possible individual reactions to different environmental stimuli. If there was a residue remaining over these primary reactions he designated this a secondary reaction. He found that nest building in the ermine-moth, Hyponomeuta, could be explained entirely on the basis of one primarily reaction, a negative stereotropism. In the formation of eating aggregations of *Hylotome ustulata,* he found primary reactions resulting from three tropisms, positive phototropism, negative geotropism, and positive stereotropism. In addition he found a synchronism in the reactions of the whole colony to the stimulus of shaking, which he believed might indicate a residue over the individual reactions and therefore be a secondary reaction. Since these caterpillars are in contact around the edge of the leaves, the means of integration is probably contact.

From the analysis of the responses concerned in the aggregating behavior of catfishes it is possible to distinguish several secondary reactions which are given by individuals exclusively to other fishes, or possibly to stimuli which have come to be associated with other fishes, and which remain as a residue over the individual reactions. These include the responses to visual stimulation given by small moving objects of about the same size and moving at approximately the rate of a small catfish; the responses of blinded fishes to vibrations set up by other passing fishes; and the responses to pressure and some chemical stimuli. All of these are not species specific. The most exclusively social reaction is the reciprocal pressure given to one catfish by another. Whether different species of Ameiurus will react in a reciprocal fashion to each other is still to be experimentally tested.

The most extensive classification of animal groupings has been made by Deegener (1918) who differentiates his two main divisions on the basis of value accruing to the individuals from the group. Those aggregations with no determinable value he terms accidential associations while groups with some value to the individual are termed societies or essential aggregations. The determination of the presence or absence of value is a difficult one as Deegener recognized. In a later paper (1919) he adds a third division, instinct societies, to include associations where the only value accruing is a satisfaction of the social instinct. This psychic factor Deegener considers of great importance. One aspect of it is what he calls "national feeling" which he believes he has demonstrated for certain arthropods. He found (1919) that mixed families of young of the same species of the arachnid Epeira, remained together in as peaceful a state as a single sympaedium. When families of different species were mixed, however, they separated out in a short time. This illustrates the "national feeling" within a species. In larvae of *Malacosoma castrense* a group which had undergone the first moult separated from one which had not, but the same groups after the moult of the younger individuals mixed freely. In this species Deegener has shown by experimentation that the sense of feeling received through the hairs of the body is very acute, but he still thinks this is not sufficient to explain the behavior observed, and that there is a psychic element present.

The aggregations of young catfishes are clearly societies according to Deegener's system, since there is an evident protective value, as well as a social value shown by the satisfaction of a contact appetite. While the young remain in the nest with the father they form with the male a primary patropaedium. When they leave the nest they form at first a primary sympaedium as long as members from the same family remain together. But since scattering occurs in the dark the aggregations are usually secondary sysympaedia, made up of individuals from several families which have come together secondarily. These groups are homotypic and reciprocal since all members of the aggregation benefit from the grouping. Groups which have been brought into the laboratory from different collecting grounds in the same locality will fuse readily, and the same is true of groups which have been kept apart in the laboratory for some time. This is true even when there is considerable variation in size between the individuals of the two groups provided only that both groups are still actively aggregating. There is therefore no evidence of what Deegener calls the "national feeling" within different groups of this species. No work has been done with other species of catfishes. Schultz (1926) in a study of a similar type of aggregation in young sticklebacks concludes that they are held together by a "necessity for association." It seems possible that with analysis a more concrete cause might be found in this case also.

That physiological state has an important bearing upon aggregations in different groups of animals has been extensively demonstrated. In an analysis of a large aggregation of the stream isopod, *Asellus communis* Say, found in nature Allee (1929), found that differences in activity and reaction habits correlated with differences in metabolic rate and associated with sex caused a great preponderance of males in these aggregations. He had previously shown (1912) by laboratory experiments that breeding conditions markedly affect the rheotaxis of this species. On the other hand bunching itself has been found to be a factor in determining the metabolic rate of animals. Thus isolated, starving Ophioderma (Allee, 1927) when compared with groups show a higher initial respiratory rate, a more rapid decrease, and a much lower final rate.

In the case of catfishes Eddy (1925) has shown that increasing or decreasing the metabolic rate by means of stimulants or depressants accordingly intensifies the closeness of aggregations or scatters them. Determinations of oxygen consumption (Eddy, 1926) showed that individuals from an aggregation have a higher rate of respiration than isolated individuals. Fishes isolated from an active group have a higher rate than individuals found in natural isolation.

The metabolic rate of young animals is generally higher than that of older individuals which would naturally give rise to greater activity, other conditions being equal. This finds a parallel in the fact that aggregations of young more active catfishes satisfy a contact appetite in motion while the more sluggish adults satisfy this appetite by often resting together. Fishes not aggregating in the aquarium will invariably aggregate when stimulated by a strong light. Such behavior seems to be the result of increased metabolic rate. These facts indicate that active aggregations and high metabolic rate are closely correlated. What the effect of resting aggregations has upon the respiration of the individuals concerned has not been determined.

A somewhat similar relation between stimulation and aggregation apparently exists among grasshoppers from the facts cited by Uvarov. Grassé (1923) has shown that reflex reactions of Acrididae, even of the non-gregarious species, are more energetic if several individuals are subjected to the same experiment together. Temperature is a very important factor in such cases as the movement of bands often occurs with rises in temperature, Sviridenko (1924) describes how swarms disperse after the final moult, and how at the same time non-social stimuli produce less response in the adult than in immature grasshoppers. Uvarov believes that reactions of solitary grasshoppers to stimuli do not differ greatly from those known in the gregarious locusts, except that there is no accumulation of nervous tension which leads to mass movements in them.

From the present investigation it is possible to draw certain conclusions concerning the behavior of young catfishes during the daily cycle. In the

evening as soon as it begins to grow dark the catfish minnows separate and swim about sweeping through the water or along the bottom with their barblets. With the highly developed taste buds over the entire body and the acute sense of touch, eyesight is, as Herrick (1902) has concluded, unimportant for feeding. Blinded fish exhibit activity similar to this in the light. The supposition that night is the normal feeding time is supported by the fact reported by fishermen that catfish and bullheads begin to bite at dusk. As soon as it starts to grow light in the early morning the young fishes come together into aggregations and remain thus during the entire day, reforming in a short time if they are scattered by a disturbance. Some feeding may occur while the fishes are aggregated but it is doubtful if this is true to any extent. Usually they are in a close group, actively pushing against each other or resting at the surface in contact or close proximity.

The comparison of the type of grouping found among catfishes with the schooling common among many species of pelagic fishes is an interesting one. Parr (1927) on the basis of much observation and some experimentation upon several species of schooling fishes has found a satisfactory explanation of this phenomenon in the assumption of a simple automatic "attraction" toward a perceived companion. This "attraction" consists of two simple antagonistic responses, a bending of the course toward the individual and an adjustment of its position to one parallel to that of the companion. These two reactions permit an explanation of the normal forward moving schools and also the milling phenomena. He concludes: "By the recognition of such tropisms or set of automatic responses to the separate perceptions of single individuals only, all peculiarities of the normal school may be satisfactorily explained without necessitating the assumption of deliberate purposeful activities or general 'social instincts,' giving complicated responses to stimulations from the entire school as a unit." By experimentation he has shown that these reactions are not given by blinded fishes, and so concludes that visual responses are the only ones concerned. He advances the supposition that the schools disperse at night, as has been concluded in the case of catfishes from this investigation. He cites the case of the catfishes and the bullheads as a probable exception to the importance of eyesight in aggregations. Because of the compactness of the schools, the highly developed sense of touch, and the poor eyesight, he believes that the tactile sense may here be the important one for aggregations with eyesight probably playing a secondary role. He has underestimated the importance of eyesight in catfishes, but is correct in his estimation of the importance of tactile responses.

Parr considers the reactions to visual stimuli which are involved in the schooling of fishes as inherited instincts, which in the light usually surpass the feeding and breeding instincts. It is quite possible similarly to conclude in the case of the catfishes that responses to contact with those to visual stimuli, either inherited or acquired by conditioning, overcome during the day

the feeding reactions in the young fishes. All of the pelagic fishes which Parr has observed have exceptionally large eyes and he believes these may be responsible for the importance of vision in their behavior. This suggests to one's mind the situation among the insects where exceptionally large eyes result in a definitely directed tropistic response to light in many species. In these cases responses to usual stimuli seem to dominate the individual. In catfishes, we have a group of fishes with unusually small eyes for this class. These eyes are functional as shown clearly by reactions to visual stimuli, but it is doubtful if these responses are as important as in other groups of fishes. The catfishes are, however, unique in their lack of scales and in the abundance of touch and taste organs situated all over their bodies and with specially developed barblets for the perception of tactile and gustatory stimuli. Their aggregations are of a type based upon contact responses, and it seems possible that these may dominate other behavior. Their reactions are complicated, as vision also plays an important role; but, in addition pressure, taste, and vibratory stimuli are also concerned.

The possible method of evolution of such social reactions as occur among catfishes is not difficult to conceive. Parr notes that all of the schooling pelagic fishes are defenseless. The catfishes have an efficient defense mechanism in the sharp spines of the fins with which they can inflict a considerable wound. But aside from this they are unusually defenseless and a desirable prey. Their bodies are soft and scaleless and the usual swimming of the young is performed slowly and steadily. An obvious protective value of the aggregating behavior is evident if one attempts to catch one fish from a group. The rapid scattering makes it impossible to keep one individual in view. The slippery bodies increase the difficulty. A single fish, however, is an easy prey. During the night this group protection is not needed as far as animals hunting by sight are concerned. Their dark color renders catfishes inconspicuous, and their highly developed tactile organs must give instant warning of any foreign body. In the laboratory blinded fishes at any sudden contact give a rapid avoiding reaction which is probably typical for normal fishes in the dark. In the light the many eyes of the group compensate for the rather poorly developed eyes of the individual. The sudden movement of one fish induces movement among all the members of the group, resulting in dispersal and escape. Thus these groups have made a beginning of a division of labor. Such protective attributes of bunching may well serve as the mechanism of natural selection by which the aggregating behavior has appeared. In the case of the pelagic fishes it is a sight reflex complex which has evolved. In the catfishes, where a group of responses concerning several sense organs is at work with conditioning possibly playing a large part in the individuals' reactions, a strong positive contact appetite may have been the factor selected. So far as known the catfish type of aggregation that has been described and analyzed is peculiar to the silurids, and appears to be associated

with the highly developed sense organs which are important here. The question of phylogenetic versus ontogenetic development of the social instinct in these fishes awaits experimental evidence.

In addition to the survival value of aggregations due to protection as suggested, there are other possibilities, evidence for which is less apparent on the surface but which have been demonstrated already for a large number of species. These are the beneficial effects of crowding which are being shown to exist for an increasing number of species and in an extended variety of conditions. A large amount of this work has been carried on by Allee and his students in this country and by Drzewina and Bohn in Europe. Experiments to test out this aspect of catfish aggregations have not been made as yet, but it seems quite possible that some protection of this kind may be present here.

Finally, in these societies of young catfish as in other sympædia the impulses leading to aggregations are entirely divorced from sex drive or sexual significance. These groups consist of sexually immature young catfishes whose social appetites are diffuse but based primarily upon reciprocal positive contact responses. Such a basis for social life is not very different from the tropholaxis which Wheeler (1923) believes is the foundation for the societies among the social insects. The importance of this contact factor is also consistent with the number of aggregations, especially among the simpler animals, which are integrated entirely by contact with distance perceptors playing no part.

SUMMARY

The young of *Ameiurus melas* (Rafinesque) give evidence of a positive response to highly conditioned water to which they have become adapted when this is run against unconditioned water in a gradient tank. They do not respond, however, to any moderate amount of conditioning, even when this is much greater than any amount which could be acting in producing in aggregations. The cutting of the olfactory nerves has no effect upon the positive reactions to other fishes in either blinded or normal individuals. A chemical factor which could be perceived through the olfactory organs is therefore probably not concerned in aggregating behavior.

Vision is an important factor in the integration of these aggregations. Neither blinded fishes nor normal fishes in the dark ever aggregate, and normal fishes follow a small moving object in a way which, if continued, would result in aggregation formation.

An individual when blinded responds positively in a large percentage of cases to another fish in motion at a distance of less than two inches, probably by detecting the vibrations set up by the tail of the other fish. This reaction is not affected by the destruction of the lateral line organs, but is almost eliminated when the skin is anaesthetized with magnesium sulphate.

To actual contact catfish give a positive response involving some ability to discriminate but not between fishes of their own and another species. Responses to contacts which probably involve both gustatory and thigmotactic stimuli received through the skin may be the primary factor underlying the social appetite which results in aggregations in this species.

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AN EXPERIMENTAL STUDY OF THE WATER RELATIONS OF SEEDLING CONIFERS WITH SPECIAL REFERENCE TO WILTING

By

ROBERT MARSHALL

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AN EXPERIMENTAL STUDY OF THE WATER RELATIONS OF SEEDLING CONIFERS WITH SPECIAL REFERENCE TO WILTING*

INTRODUCTION

The present paper reports the results of a limited study on the physiological ecology of conifer seedlings. The study was undertaken as a piece of physiological research with an ecological viewpoint, but the species of plants used and the general plan followed will perhaps make it of value to those who are interested in forest ecology, as the results reported deal with the water relations between plant health and soil-moisture conditions for some representative conifers during the seedling stage.

Many investigators have taken part in developing present day knowledge of plant wilting as related to soil-moisture conditions. Sachs ('59) allowed tobacco plants to wilt in soils of different texture and showed clearly that the amount of water remaining in the soil at the time of wilting was related to the size of the soil particles; the coarser the soil, the smaller the amount of water left. At about the beginning of the present century Heinrich ('94), Gain ('95), Hedgcock ('02) and Clements ('05) measured the amount of water remaining in the soil when plants of several different kinds had wilted, and concluded that drought resistance might be estimated in terms of the soil-moisture content when wilting occurs. Many investigators have studied this so-called non-available water content, which Clements has called the echard. Briggs and Shantz ('11, '12, '13) carried out an extensive series of experiments which have greatly influenced subsequent progress of knowledge along these lines. They presented more data than had ever been available before and improved technical methods. They studied twenty different types of soil, more than a hundred species of plants, and made in all more than 1,300 separate determinations of soil-moisture corresponding to what they termed permanent wilting of the plants. On the basis of the ideas introduced by these writers, and with due reference to further developments by other students of water relations, permanent wilting may be defined as that stage in the progressive wilting of a plant at which the rate of water absorption through the roots becomes so slow that, even though there is no further water loss through transpiration, recovery of turgor is impossible without increasing the water content of the soil. Thus the amount of water left in the soil at the beginning of permanent wilting becomes a serviceable measure of what the earlier writers termed non-available soil moisture. Briggs and Shantz came to the somewhat startling conclusion that all plants were capable

* Botanical Contribution from the Johns Hopkins University; No. 112.

of drying a given soil to practically the same extent, being thus in pronounced disagreement with most earlier experimenters, who had thought that species differed with respect to their capacity for removing water from the soil. It is true that Cameron and Gallagher ('08) had previously suggested a similar generalization. Briggs and Shantz found, however, that the amount of water left in the soil at the beginning of permanent wilting differed greatly for different soils, and concluded that it was directly related to the moisture equivalent. This soil characteristic, as defined by Briggs and McLane ('07), is the residual water retained by the soil after it has been subjected under standard conditions to a centrifugal force equivalent to 1,000 times gravity. Because of an apparently constant relationship between the moisture equivalent of the soil and the amount of water remaining at the beginning of permanent wilting in their experiments, Briggs and Shantz presented formulas to give what they called the wilting coefficient of the soil, which was thus regarded as a soil characteristic.

Several subsequent writers have questioned the conclusion that the nature of the plant has no bearing on the soil-moisture content at the beginning of permanent wilting. Crump ('13) attributed these results of Briggs and Shantz to the prevalence of agricultural crops among the material with which they worked, to the particular conditions of their experiments (especially the low transpiration rates prevailing), and to the difficulty of determining just when permanent wilting sets in. Wilson's studies ('27) indicated that different kinds of plants may enter permanent wilting with markedly different soil-moisture residues. Maximov ('29), on the other hand, seems to consider the preponderance of evidence as in agreement with Briggs and Shantz. Brown ('12), Caldwell ('13), and Shive and Livingston ('14) all presented evidence indicating that the residual moisture in the soil at the time of wilting varied not only with the soil texture but also with the atmospheric environment, the greater the evaporating power of the air the greater the moisture content of the soil when the plants entered permanent wilting. Recent studies of Veihmeyer ('27) and Veihmeyer and Hendrickson ('27, '28) seem to support the earlier conclusions of Briggs and Shantz in this regard. Several investigators, notably Brown ('12) and Thom and Holtz ('17), pointed out that the conditions under which plants had been grown has also influenced the amount of residual soil moisture at the onset of permanent wilting. Plants grown in moist soil left more water in the soil when water absorption practically ceased than was left by plants grown in drier soil. Harris ('14) and Jean and Weaver ('24) observed that "the general effect of decreased water content of the soil, providing enough is available to produce moderate growth, is to accelerate root development in relation to growth of tops."

It is apparent that general agreement has not been reached with respect to this vexed question of the relation of wilting to soil-moisture content. A sort of dynamic approach to this important question was suggested even be-

fore the time of Briggs and Shantz's work, when Livingston ('06) used Pfeffer osmotic cells as standard water absorbers (like artificial roots). The same writer presented some observations on the capacity of a soil to deliver water to the supernatant air through ordinary evaporation. The osmometer method for studying soil-moisture conditions was later improved by Pulling and Livingston ('15) and by Pulling ('17), but it did not appear to be very promising. Dry porous porcelain was introduced as a water absorber by Livingston and Koketsu ('20), who first employed the porcelain cones called soil points. These, like the osmotic cells mentioned above, imitate plant roots in that they remove water rapidly from the soil in which they are placed. With some improvements, soil points have been used by Thone ('22), Livingston and Ohga ('26), Livingston, Hemmi, and Wilson ('26), Wilson ('27), Baldwin ('28) and others. Using the same general principles, and at Livingston's suggestion, Mason ('22) and Hardy ('23, '24) employed as water absorbers ordinary lead pencils sharpened to uniform conical points by means of a mechanical sharpener. These experimenters were no longer studying the static water content of the soil and its fluctuations. They turned attention to the dynamic water-supplying power of the soil—in other words, the rate at which water was available to the absorbing surface of the plant. The aim of those employing the soil-point method has been similar to that for the attainment of which osmometers had been tried; namely, to measure the capacity of the soil to deliver water to a suitable standard water-absorbing surface. The soil-point method will be considered in some detail further on. It at least offers great promise for dynamic soil-moisture studies. It permits the comparison of soil-moisture-supplying power with the water-absorbing power of the plant and with the plant's turgor or stage of wilting, and it makes such comparisons possible without any reference to the coarseness or fineness of the soil or its state of packing. Soil-point readings at the onset of permanent wilting have been found to be the same, or very nearly the same, for soils of different degrees of fineness, at least when the same kind of plant in the same developmental stage and with the same climatic complex is employed for the tests.

As has been suggested, one great difficulty has always beset students of plant wilting, that of ascertaining just when a plant is wilted to any specified degree. Wilting is, of course, a progressive process and cannot be sharply separated into distinct phases, but, following Livingston ('17), four stages may be outlined as follows: (1) Incipient drying—the usual fluctuating water deficit due to tissue water contents somewhat below the water content at maximum turgor. (2) Temporary wilting—a continuous series or range of wilting stages from which the wilted tissues may recover if transpiration is subsequently decreased and remains low for a sufficient time. (3) Permanent wilting—the series of stages from which plants cannot recover unless the rate of water supply to the roots is increased. (If permanent wilting proceeds far enough death supervenes.) (4) Post-mortem desiccation. The

onset of any specific stage of progressive wilting is in most instances, very difficult to ascertain by eye. It is only rarely that the experimenter has as satisfactory material as Koketsu's ('25) Mimosa. In tender herbaceous plants it is in many cases possible to observe ocularly the beginning of temporary wilting and the onset of some subsequent stages. When it comes to such plants as conifers or cacti, however, it is almost or quite impossible to decide by direct observation just when any stage of wilting has begun. Briggs and Shantz ('12) suggested that permanent wilting in a cactus might be considered as beginning when the plant had lost 1 per cent of its original water content. Different tissues of any wilting plant do not lose in water content at the same relative rates, however. As Livingston ('12) has pointed out, many of the leaves of a plant may have dried enough to be killed while some other parts may still be highly turgid and even growing. MacDougall, Long and Brown's ('15) Echinacactus that received no external water for several years had lost a large part of its original water but it persisted in producing flowers and fruits at the usual season. Nevertheless, the suggestion of Briggs and Shantz leads to the thought that different degrees or stages of decreased turgor or of progressive wilting might be identified in terms of the relative water contents of the tissues considered.

Many observers have found that plant water content fluctuates remarkably, even in a single day. Livingston and Brown ('12) specifically pointed out that the diurnal minimum of foliar water content furnished a criterion by which the approach of the beginning of temporary wilting might be detected. In Bakke's ('15, '18) experiments on the diminishing transpiring power of wilting leaves he found that the beginning of permanent wilting appeared to correspond to a temporary increase in transpiring power. He measured transpiring power by the method of the standardized hygrometric paper devised by Livingston ('13) and improved by Livingston and Edith B. Shreve ('16). Koketsu ('26) studied the progress of wilting with reference to changes in the transpiration rate. He says: "During the process of wilting the index of the transpiring power itself decreases progressively until it reaches its minimal value at the critical point of wilting. After that time, it begins again to rise more or less, and then the plant falls into the drying phase of low transpiration or evaporation."

The foregoing paragraphs are not intended to be a review of the extensive literature of the soil-moisture relations of plants. They may serve, however, to illustrate some of the main considerations from which further research needs to start. In the present study of wilting and drying in young conifer seedlings, the changing appearance of the seedlings was observed and their diminishing water content was measured. An ocular criterion of the beginning of permanent wilting was employed, but it was unsatisfactory and water content appeared to furnish a more definite criterion. The water-supplying power of the soil at the time the seedlings entered a rather definite stage of wilting was ascertained.

The experiments described in this paper were carried on at the Laboratory of Plant Physiology of the Johns Hopkins University, where equipment and facilities were freely furnished. The author wishes to acknowledge indebtedness to the pioneer publications by Bates and Zon ('22), Bates ('23), and Pearson ('24). Especially he wishes to express his appreciation for very essential advice, guidance, and criticism received from Professor Burton E. Livingston, Director of the John Hopkins Laboratory of Plant Physiology.

Experiments

The seedlings.—The seedlings for this study had been grown from seed in rectangular boxes of soil in a greenhouse room. These boxes were of galvanized sheet iron, 30 cm. wide, 55 cm. long and 20 cm. deep. The soil mass was about 15 cm. deep in each box, including a bottom layer of cinders 2 cm. deep. Only one kind of seed was planted in any box. The soil was kept moist by frequent watering throughout the period between planting and the beginning of an experiment, which occurred about 24 days after the appearance of the plantlets above the soil surface. The whole period from the time of planting covered 34 to 60 days, varying with the time required for germination. Some species appeared above the soil in only 10 days; others, after more than a month. Throughout the period of watering the water-supplying power of the soil at a depth of from 3 to 5 cm. generally fluctuated roughly between 150 and 250 mg. for the first hour of absorption, as read on Livingston soil points. This corresponded to a volumetric water content between about 5.2 and about 7.1 per cent. At the end of the preparatory period watering was discontinued and observations on water content of tops were immediately begun and continued throughout the subsequent drying period.

The soil used was a nearly pure, white quartz sand with a water-holding power of 36.4 per cent on a volumetric basis, or 26.0 per cent on the basis of dry weight. These values were secured by means of the Hilgard ('12) method with a 1-cm. column of soil. Oven-dry, without packing, this sand weighed 1.95 g. per cubic centimeter.

As a guide in the placing of the seeds on the leveled soil surface a piece of galvanized wire netting with square meshes about 8 mm. in diameter was laid on the soil and one seed was placed in the center of each opening. The seeds were thus regularly spaced about 8 mm. apart in each direction, 1,500 seeds being uniformly distributed over the seed bed, which was 30 cm. wide and 55 cm. long. After being placed the seeds were covered with sand, regularly to a depth of 7 mm. but to a depth of 10 mm. for the unusually large seeds of western yellow pine and slash pine. A suitable wooden block supported on the opposite margins of the box and easily slid from one end of the seed bed to the other was used as a gauge to smooth the soil at the proper level before the seeds were planted and another block was used similarly after the sand cover had been applied.

The twelve species of coniferous trees studied are listed in Table I. Nine experiments were performed with Norway spruce, 6 with Norway pine and 1 with each of the other species. The necessary supplies of seed were kindly furnished by the United States Forest Service, the New York State Conservation Commission, and the Louisiana Department of Conservation. The writer is gratefully appreciative of the kindness of Raphael Zon, Julius F. Kümmel, D. S. Olsen, E. L. Demmon, L. J. Pessin and W. G. Howard, who helped in procuring seeds. Some special data concerning the lots of seed used are given in Table I.

In order that all seedlings used in an experiment should have developed under similar conditions and should be of nearly the same age, measured from the time of their appearance above the soil, seedlings that germinated early or late were removed from the beds before the beginning of an experiment and those used were all of nearly the same age, from about 21 to 27 days. The range of ages in any one experiment was usually only 7 days, although, with a few beds it was necessary to allow this range to be as much as 13 days. Ages (from the time of appearance above the soil) are shown by the dates given in Table II, along with average stem height and root length for the beginning of each experiment. Very uniform stands of seedlings

TABLE I. *Seed data.*

Species	Year of Crop	Source	Altitude of source above sea level	Average weight per seed	Times of planting
			Feet	*Mg.*	
Abies grandis Lindl. (Lowland white fir)	1929	Montana	3,000	18.9	Jan., 1930.
Picea excelsa (Norway spruce)	1928	Norway	.....	7.4	Mar., Oct., Nov., 1929; Jan., Feb., 1930.
Picea glauca B. S. P. (White spruce)	1928	Ontario	.....	3.7	Mar., Oct., 1929.
Pinus caribaea Morelet. (Slash pine)	1928	Georgia	.....	28.0	Oct., 1929.
Pinus echinata Mill (Shortleaf pine)	1928	Louisiana	.....	9.1	Oct., 1929.
Pinus monticola D. Den. (Western white pine)	1929	Idaho	3,000	14.6	Dec., 1929.
Pinus ponderosa Laws. (Western yellow pine)	1927	Montana	4,500	52.2	Oct., 1929.
Pinus resinosa Sil. (Norway or red pine)	1927	Wisconsin	.....	6.3	Mar., Oct., Nov., 1929; Jan., 1930.
Pseudotsuga taxifolia Britt. (Douglas fir)	1928	Washington	500	12.5	Oct., 1929.
Thuja occidentalis L. (Northern white cedar)	1928	New York	.....	1.2	Mar., Oct., 1929.
Thuja plicata D. Don. (Western red cedar)	1929	Montana	3,000	0.9	Jan., 1930.
Tsuga heterophylla Sarg. (Western hemlock)	1929	Idaho	2,500	0.8	Jan., 1930

TABLE II. *Dates of experiments and heights of plants when experiments began; that is, when watering was discontinued.*

Species	Exp. No.	Average Date of first Appearance above soil	Beginning of Experiment	Stem Height	Root Length	Occurrence of critical wilting	End of Experiment
				Cm.	*Cm.*		
	1	May 3, '29	May 27, '29	4.0	2.3	June 3, '29	June 4, '29
	2	Nov. 8	Dec. 4	3.3	4.2	Dec. 9	Dec. 22
	3	Dec. 15	Jan. 12, '30	3.9	3.8	Jan. 17, '30	Jan. 25, '30
	4	Dec. 15	Jan. 12	3.7	3.5	Jan. 18	Jan. 25
Norway spruce	5	Jan. 23, '30	Feb. 20	3.2	4.8	Feb. 23	Feb. 28
	6	Feb. 23	Mar. 20	3.0	4.0	Mar. 24	Mar. 31
	7	Dec. 15, '29	Feb. 13	3.8	5.8	Feb. 18	Feb. 27
	8	Nov. 8	Jan. 15	3.3	6.0	Jan. 22	Jan. 23
	9	Nov. 8	Feb. 13	3.4	8.6	Feb. 22	Mar. 4
	10	Nov. 12	Dec. 6, '29	3.4	4.1	Dec. 14, '29	Dec. 22, '29
	11	Dec. 20	Jan. 15, '30	3.3	3.7	Jan. 23, '30	Feb. 4, '30
	12	Dec. 20	Jan. 15	3.4	3.8	Jan. 24	Feb. 4
Norway pine	13	Jan. 27, '30	Feb. 23	3.2	4.9	Feb. 28	Mar. 4
	14	Dec. 20, '29	Feb. 13	3.5	6.2	Feb. 24	Feb. 28
White spruce	15	Nov. 15	Dec. 10, '29	3.3	3.4	Dec. 15, '29	Dec. 21, '29
Northern white cedar	16	Nov. 17	Dec. 12	1.9	2.8	Dec. 18	Dec. 20
Shortleaf pine	17	Nov. 14	Dec. 9	2.8	5.1	Dec. 18	Dec. 18
Slash pine	18	Nov. 15	Dec. 10	5.0	6.3	Dec. 18	Dec. 18
Western yellow pine	19	Nov. 18	Dec. 12	3.4	7.0	Dec. 19	Dec. 21
Western white pine	20	Jan. 18, '30	Feb. 13, '30	3.7	7.0	Feb. 21, '30	Feb. 21, '30
Douglas fir	21	Nov. 22, '29	Dec. 15, '29	3.1	5.4	Dec. 21, '29	Dec. 22, '29
Lowland white fir	22	Feb. 3, '30	Mar. 1, '30	3.5	5.4	Mar. 7, '30	Mar. 13, '30
Western red cedar	23	Jan. 25	Feb. 20	1.7	4.3	Feb. 25	Feb. 28
Western hemlock	24	Feb. 11	Mar. 7	1.6	1.4	Mar. 11	Mar. 13

were usually secured by this method. Whenever the seedlings of a sample were removed from a bed for the determination of the water content of tops they were selected by eye to be representative of the entire stand and the few seedlings in any stand that were unusually large or small were not used in the samples. Heights were measured from junction of root and stem to insertion of cotyledons. In Table II the dates of the first appearance of the seedlings above the soil, are shown for each experiment the dates of beginning and end of the experiment, and the date of critical wilting.

Atmospheric Conditions and Radiation during Experiment Periods.—The atmospheric conditions of the experiments differed more or less. Continuous records of air temperatures were obtained from a shaded thermograph. Daily atmometric records were made from two standardized white porous-porcelain atmometers, corrected to the Livingston ('15) standard for white spheres, the readings being taken at 9 o'clock each morning. The drying effect of radiation was approximated for each day by means of a Livingston radio-atmometer ('11), consisting of two atmometer spheres operated side by side, one white and the other blackened with a collodion-lampblack film (Livingston and Wilson, '26). The index of radiation effect is taken as the difference between the corresponding corrected readings from the two spheres. The climatic records are summarized in Table III. They may be useful as showing at least some important features of the climatic influences that prevailed during the experiment periods.

Measurement of Water Contents of Seedling Tops.—The moisture contents of representative seedlings, excluding the roots, were measured at the beginning of each experiment and at frequent intervals thereafter until a critical moisture-content value had been passed, and in many cases until the seedlings were dead and nearly air dry. These measurements were made by sampling. For each sample 10 representative seedlings were taken from the bed, their roots were removed and the remaining top portions were then placed in a closed chamber, from which they were transferred one at a time for the first weighings. After each individual had been weighed it was placed upright in a carrying rack; a horizontal perforated disc of sheet metal supported on an evaporating dish. The tiny stems were inserted from above, one in each perforation, with the leaves resting on the disc. Each sample had its own rack. The use of the racks made it easy to keep the several individuals of a sample separate, without risk of interlocking of the needles. After the first weighings had been made any tops that deviated in weight from the average of the ten by more than 25 per cent were discarded. Not more than three were thus thrown out as irregular in any case and the whole 10 were usually retained. The average green weight per top for the "corrected" sample was taken to represent the average green weight of the tops of the whole population of the bed from which the sample had been taken. The general consistency of the results indicates that this method of

TABLE III. *Records of climatic conditions in the greenhouse during the experiments.*

Date	Maximum shade temperature	Minimum shade temperature	Atmometer loss (white sphere)	Atmometric index of solar radiation
	Deg., F.	*Deg., F.*	*Ml.*	*Ml.*
May 2, '29	..	..	3	0.5
3	..	..	12	0.5
4	..	..	15	1.0
5	..	..	7	1.4
6	..	..	12	1.1
7	..	..	16	1.8
8	..	..	14	1.1
9	..	..	10	1.2
10	..	..	18	1.8
11	..	..	16	1.8
12	..	..	16	1.8
13	..	..	6	0.3
14	..	..	4	1.0
15	81	60	16	1.5
16	83	58	19	1.7
17	76	50	13	1.7
18	76	53	11	1.4
19	82	65	10	1.2
20	69	51	2	0.4
21	66	50	7	0.9
22	78	50	24	2.6
23	78	49		
24	80	55	12	1.8
25	84	57	15	1.1
26	81	56	12	1.7
27	78	61	9	0.9
28	89	64	8	1.1
29	81	65	5	1.0
30	86	63	11	1.4
31	96	64	15	1.6
June 1	91	64	21	1.2
2	80	56	16	0.9
3	..	..	12	0.7
4	..	..	15	1.6
5	..	..	13	1.3
6	83	57	12	1.0
7	90	66	17	1.1
8	70	56	4	0.0
9	71	54	11	1.4
10	75	56	15	1.7
11	89	52	..	...
Nov. 8	77	..	13	0.3
9	85	62	24	2.4
10	80	55	16	1.5
11	81	63	20	2.0
12	82	64	13	0.7
13	74	66	5	0.6
14	78	70	7	0.3
15	77	61	12	1.2
16	87	59	27	0.8
17	76	64		
18	..	..	12	1.0
19	78	61	18	1.5
20	82	56	11	0.9
21	79	54	24	1.3
22	81	55	12	0.2
23	74	58	14	0.0
24	75	53	11	0.1
25	77	51	17	0.4

TABLE III *(continued)*.

Date	Maximum shade temperature	Minimum shade temperature	Atmometer loss (white sphere)	Atmometric index of solar radiation
	Deg., F.	*Deg., F.*	*Ml.*	*Ml.*
Nov. 26, '29	78	56	18	2.2
27	84	61	22	1.6
28	78	59		
29	83	54	73	5.7
30	83	44		
Dec. 1	80	47	..	...
2	75	52	12	0.0
3	82	63	19	0.6
4	81	50	18	0.5
5	82	54	20	2.7
6	84	58	22	2.4
7	81	57	17	0.7
8	82	64	20	2.1
9	80	57	18	1.2
10	80	59	16	1.2
11	74	60	14	0.3
12	77	49	12	0.0
13	87	60	14	0.3
14	92	72	17	1.0
15	89	69	19	1.2
16	84	70	15	0.2
17	89	65	14	0.0
18	84	72	10	0.4
19	84	76	12	0.6
20	87	59	23	1.0
21	87	55	22	1.5
22	83	59	14	0.0
Jan. 12, '30	..	..	20	0.0
13	..	..	14	0.2
14	86	..	11	0.0
15	86	71	14	0.4
16	82	62	16	0.5
17	83	54	19	1.0
18	78	57	17	0.0
19	88	56	22	2.0
20	81	50	18	0.6
21	83	61	16	0.2
22	86	54	15	0.0
23	94	51	20	1.0
24	81	51	25	3.0
25	85	50	45	2.8
26	80	56		
27	90	63	12	0.3
28	83	..	36	0.7
29	86	55		
30	81	56	14	0.1
31	88	50		
Feb. 1	86	56	86	4.6
2	85	62		
3	85	67		
4	84	..		
5	88	64		
6	81	60		
7	87	57		
8	89	56	196	7.8
9	80	59		
10	82	59		
11	83	54		
12	93	60		

TABLE III *(continued)*.

Date	Maximum shade temperature	Minimum shade temperature	Atmometer loss (white sphere)	Atmometric index of solar radiation
	Deg., F.	*Deg., F.*	*Ml.*	*Ml.*
Feb. 13, '30	88	59		
14	86	58	52	1.6
15	78	61		
16	84	44	23	0.9
17	87	47	19	0.7
18	94	58	27	0.6
19	95	63	31	1.5
20	101	71	36	1.2
21	94	70	35	1.0
22	87	65	24	1.0
23	80	65	14	0.6
24	88	62	23	1.6
25	91	72	33	2.2
26	79	64	13	0.4
27	88	56	22	1.4
28	93	56	28	1.4
Mar. 1	86	56		
2	75	56	54	2.5
3	78	50		
4	80	..	46	2.8
5	85	50		
6	..	..	26	1.0
7	..	..	56	4.5
8	..	..		
9	84	58	25	1.9
10	84	59	24	1.8
11	71	60	29	2.6
12	79	55		
13	83	56		
14	85	60	112	6.2
15	81	52		
16	88	53		
17	88	60		
18	87	71	89	7.0
19	81	64		
20	84	55	28	1.6
21	86	59	28	1.7
22	87	47	25	1.6
23	89	48	26	1.8
24	79	51	21	2.1
25	79	..	13	0.9
26	79	60	17	2.1
27	77	56	21	1.6
28	80	53	21	1.8
29	82	54	38	2.6
30	79	55		

sampling was generally adequate to give values fairly representative of the stands.

After the first weighing each rack with its seedling tops was transferred to an electrically controlled oven with maintained temperature of 105°, where it remained till loss of weight had ceased. The length of the drying period was regularly never less than 4 hours and never more than 12 hours. The racks

were then transferred to desiccators with dried calcium chloride, for cooling and transfer to the weighing room, where each seedling top was again weighed. The average dry weight per top was finally computed for each sample and the difference between the average green weight and the average dry weight was taken to represent the average moisture content of the seedlings in the bed in question at the time of sampling. All weighings were made to the fourth decimal place by means of an ordinary rider on the balance beam. The balance case was provided with a beaker of dried calcium chloride for the dry-weight weighings.

Early in the progress of the experiments it was found that the moisture content of the seedling tops fluctuated in a regular way with the alternation of day and night, being relatively high at night and relatively low in the daytime, as has been observed in a number of instances for other kinds of plants. This diurnal fluctuation rendered it necessary that the regular moisture-content samplings described above should be made in the very early morning, when the moisture content was nearly at its maximum and before the occurrence of the morning rise in transpiration rate. The work of sampling began between about 3:30 and 6 o'clock, early enough in every case so that all green-weight weighings for the day might be completed before sunrise. The number of these weighings to be made on any morning varied from 10 to 90.

Measurements of Soil-Moisture Conditions.—The initial water-supplying power of the soil in each seed bed was measured for a depth of 3 to 5 cm., at the time of the occurrence of a certain advanced stage of wilting in the plants, to be characterized below. Livingston soil points were employed. They were hollow porous-porcelain pieces, each with an upper section of cylindrical shape (2.5 cm. high and 2.2 cm. in diameter outside) continuous with a lower portion of conical form (5 cm. high), which terminated in a point. The whole piece was 7.5 cm. long and the wall was about 2 mm. thick. It was waterproofed outside except for an absorbing zone 2 cm. high, located on the conical part with its upper margin 3 cm. from the upper end of the piece. The area of the absorbing surface was about 12 sq. cm.

In this study the soil points were first weighed oven-dry, in tared glass test-tube-like containers with cork stoppers, one instrument in each container. They were then inserted vertically into the soil to their full length; which brought the absorbing zone into capillary contact with the soil between 3 and 5 cm. below the soil surface. After a 1-hour exposure they were lifted, quickly brushed to remove adhering soil grains and returned to their respective containers, in which they were again weighed. Weighings were to the nearest milligram. The amount of water absorbed during the 1-hour exposure in the soil (the difference between the two weights) was taken as a measure of the initial water-supplying power of the soil for the given depth. It represents the amount of water that an adequate absorbing surface

of 12 sq. cm. might take up in the initial hour of exposure to the soil mass in question, at a depth of 3 to 5 cm.

The soil points used in this study had been standardized by comparing their absorption when all were exposed for an hour, side by side and about 12 cm. apart, in a box of sand of nearly uniform moisture content. Three such calibrations were made, with sand of different water contents, absorption by a single point being about 72 ± 7 mg., 31 ± 5 mg. and 12 ± 1 mg., respectively. The deviation from the average is seen to be not far from $\pm$ 10 per cent. Deviations among a set of readings from several soil points exposed at the same time in the same box of soil are only partially due to differences in the instruments. They are likely to be higher or lower because of slightly different exposures to the surrounding soil (which is always somewhat compressed and otherwise disturbed when the instrument is inserted. Furthermore, different locations in the same soil mass are apt to differ considerably, as is generally shown when a presumably uniform soil mass is studied for water content by means of several samples taken at the same depth. The soil points themselves appear to be more nearly uniform in water-absorbing capacity (up to at least 2 gm. or more) than are the water-supplying powers of different soil locations at the same depth in the same supposedly uniform soil mass. It is of course to be remembered that plant roots must encounter just this same sort of deviation in the water-supplying power of the soil about them, as they are brought into contact with unexplored soil surfaces through elongation and the continuous formation of new absorbing surfaces.

With soils of very low water-supplying powers air-dried or even desiccator-dried soil points may fail to give satisfactory readings because their water-absorbing capacities may be too low at the beginning of an exposure. It sometimes occurs that an insufficiently dry soil point may lose water to the soil instead of taking up water from the soil, thus giving a negative reading, which may be disconcerting. When very dry soils are to be studied the instruments should be thoroughly dried and they should be inserted in the soil with little chance of their taking up significant amounts of water from the air just before insertion. For readings of 20 mg. or less some difficulties of this sort were encountered in the early stages of this study but such difficulties were finally avoided by drying all soil points at 90° for 16 hours or more. They were always allowed to cool in their closed containers before being weighed and applied to the soil.

The value of the soil-point method is dependent on the assurance that all water reaching the absorbing surface during the exposure period is absorbed very quickly. Only so long as the absorbing power of the instrument is greater than the supplying power of the adjacent soil can the amount of water absorbed in the standard exposure interval be taken as a measure of the dynamic soil feature in question. As water enters the instrument

the absorbing power of the latter decreases, of course, and this characteristic must eventually come to equal the supplying-power of the soil; after which the rate of absorption is determined by the condition of the instrument rather than by soil influences. It is therefore necessary that the exposure period should always be short enough so that the absorbing power of the porous-porcelain surface shall still exceed the supplying power of the soil at the end of the exposure. For wet soils (with high initial water-supplying powers) the exposure should be relatively short, while it may be very long if the initial supplying power of the soil being tested is very low. These considerations have received attention by Livingston and Koketsu, and Wilson. For the low supplying-power values dealt with in the present paper in the 1-hour exposure period was surely not too long. It might have been somewhat longer, but it is desirable that all comparable readings be obtained with the same exposure. The 1-hour period has been used by most workers who employed this method and appears to be very satisfactory.

The relation of soil-point values to corresponding water contents of the soil.—Soil-moisture conditions have generally been measured and defined in terms of the weight of water held in a given volume of soil or in a volume of soil containing a given weight of non-water material. Either of these methods is apparently useful in some ways, especially in studies dealing with the molecular attractions between the non-water particles of the soil and its contained water. When soil-moisture conditions are to be related to plant health, however, neither of these water-content criteria is satisfactory, for the influential condition that requires quantitative measurement is then not the *water-content* of any unit of soil, however defined, but the *capacity* of the soil to *supply water* (*i. e.*, soil solution) to the absorbing surfaces of the root system of the plant.

Because the same type of sand was used for all the beds during this study and all had very nearly the same packing, there should be a definite relation between soil-point readings and the corresponding water-content values of the soil computed either on the basis of weight or of volume. A special experiment was performed to bring out this relation for these beds. A bed was prepared just like those used for the seedlings but no seeds were planted in it. It was thoroughly watered and was then allowed to dry out until the soil had approximately attained the air-dry condition. Daily soil-point readings were made throughout this period for a depth of 1 to 3 cm. and for a 1-hr. exposure, three instruments being employed at each observation. Corresponding daily samples of soil from the bed, to a depth of 4 cm. were also taken, by means of a 1-cm. cork borer, which removed about 3.1 ml. of soil. For each soil-point reading this sample was taken a few centimeters away from the soil point. The dry weight and water content of each sample were ascertained in the usual manner

and each water-content value was finally computed as a percentage on the basis of the volume of the sample.

When the soil of this special bed had about ceased to lose water it was thoroughly wetted again and the experiment was repeated. For the first experiment drying out took 13 days, for the second it took 14 days. The corresponding total losses from the white atmometer sphere were 169 ml. and 167 ml. The results for the two series were very consistent. These results are shown in Table IV. Each soil-point reading and the corresponding volumetric water-content value were plotted as the rectangular coördinates of a point on coördinate paper and when all points for both experiments had been marked a smooth curve was drawn to represent the rate of decrease in soil-point readings with respect to the rate of decrease in volumetric percentages of soil water content. To obtain the gravimetric values of soil-moisture percentage it is necessary only to multiply each volumetric percentage by 0.70 throughout the series.

TABLE IV. *Water-content percentages corresponding to representative soil-point readings, for a depth of 2-3 cm. in a sand bed like those used for seedlings.*

Soil-point reading	Water-content (volumetric, on basis of soil volume)	Soil-point reading	Water-content (volumetric, on basis of soil volume)
Mg.	*Per cent.*	*Mg.*	*Per cent.*
0	0.2	150	5.8
10	0.6	200	7.0
20	1.0	250	8.0
30	1.4	300	8.8
40	1.8	400	9.7
50	2.3	500	10.2
60	2.7	600	10.6
80	3.5	700	10.8
100	4.2	800	11.0

For the kind of sand and the degree of packing employed in the beds the magnitude of the initial water-supplying power is approximately proportional to the volumetric water-content percentage, up to a supplying-power value of about 125 mg. or to a volumetric percentage of about 5. For the range from approximate air dryness to this limit the soil-point reading (in milligrams) corresponding to any volumetric percentage may be approximately ascertained by multiplying the corresponding percentage by 25; that is, the initial supplying power for the 2—3 cm. depth increases by about 25 mg. for each gram of water held in 100 ml. of the upper 4 cm. of soil. Beyond a supplying power of about 125 mg. this relation of proportionality does not continue and the supplying-power value increases at an accelerated rate. A graph showing these features may readily be constructed from the data given in Table IV. It must be remembered that these special relations refer specifically to the soil and packing of the sand beds and to the depths to

which the indices refer; and that other soils, other degrees of packing or other depths would be expected to show quite different relations between the dynamic index of soil-moisture condition and the corresponding static index. As long as the nature and packing of all soils in a series are alike, as in this study, either method for computing water content is equally good, since one series of percentages can be derived from the other by the employment of a simple coefficient; but of course the coefficient varies with the kind of soil and its state of packing, and water-content values cannot be compared when soil and packing are not alike throughout a series of observations. The dynamic index of soil-moisture condition cannot in general be deduced from water-content percentage, excepting by means of an empirically derived table of corresponding values such as those given in Table IV. However, for this particular study, and only for its background complex, this table may be used if one wishes to convert soil-point readings into the corresponding percentages. It is unfortunate that these water-content percentages refer to a depth of from 0 to 4 cm., while the corresponding soil-point readings refer to a depth of from 2 to 3 cm. and the soil-point readings for the seedling beds refer to a somewhat greater depth (3 to 5 cm.), but the errors of relationship introduced by these discrepancies are systematic and probably negligible considering the deviations encountered in this kind of experimentation.

RESULTS WITH NORWAY SPRUCE

Wilting and Drying of the Plants

The results secured with Norway spruce will be presented first, after which those for the other species will be set forth somewhat more briefly. At the time when watering was discontinued the leaves of the first whorl were just beginning to appear and the six or eight cotyledons represented practically all the foliage. Turgor throughout the plant was high. The cotyledons appeared glossy, the epidermis being tightly stretched, somewhat like the skin over a strongly flexed human knuckle. In a few days the appearance of the seedlings began to show effects of inadequate water supply. The epidermis gradually became less glossy and, as turgor decreased, reflected light less thoroughly. The color of the cotyledons also became duller. Between a highly turgid cotyledon and one with a low degree of turgidity the difference is similar (for some eyes, at least) to that between paper with a glossy and paper with a matt surface. Obvious wrinkling of the leaf epidermis soon set in and became more and more pronounced. Small longitudinal grooves were observed and the general dullness was further accentuated. After a time the small grooves appeared to run together to form larger grooves, which were produced unequally on different sides of the cotyledon and at different levels. The shrinkage thus indicated resulted in a flattening and twisting of the cotyledons. Twisting began near the cotyledon tip and proceeded toward the

base. A still later phase of drying showed the cotyledons severely twisted and flattened, pale green or fawny in color and exceedingly brittle. In this last phase they crumbled when rolled between the fingers. For Norway spruce the position of the cotyledons did not alter considerably during the drying-out process and, aside from the twisting of the cotyledons themselves, the seedlings as a whole had about the same general shape when air dry as when fully turgid. More advanced seedlings, with the true leaves of the first whorl large enough to be conspicuous, showed the beginning of twisting in those leaves somewhat before twisting was observed in the cotyledons.

While these changes were taking place in the cotyledons the hypocotyls passed through a similar series of progressive degrees of wilting and withering but they did not usually become markedly twisted. For most of the other species studied wrinkling and shrivelling were apparent in the cotyledons somewhat sooner than in the hypocotyl, but for Norway spruce wrinkling of the hypocotyl was generally observable slightly before the cotyledons wrinkled.

It appears that the onset of a wilting phase similar to what Briggs and Shantz ('11) called "permanent wilting" may perhaps be denoted for these Norway spruce seedlings by the appearance of obvious epidermal wrinkles and the beginning of twisting of cotyledons. In the following paragraphs this will be called the *critical phase of wilting*. Seedlings in the critical phase soon recovered if the bed was watered but those whose cotyledons had become brittle were irretrievable. Death apparently occurred in the last stage of wrinkling and twisting, just before brittleness began to develop.

Decreasing Water Content of Seedling Tops As Drying Went On

Data of moisture content of plant tops. Table V gives the data on seedling moisture content secured from nine beds of sand-grown Norway spruce, along with the corresponding records of water loss from the white atmometer sphere. Each water-content value appears in three forms. First is given the average of the actual water contents of all the tops in the "corrected" sample, as 29.9 mg. for May 27 in Experiment 1. Then follows the corresponding actual average of the water-content percentages of all tops in the corrected sample. Each individual water-content is expressed as a percentage of the corresponding dry weight and all these percentage values for the corrected sample are averaged to give the value shown, as 396 per cent. for May 27 in Experiment 1. Finally, this average percentage for each observation is expressed in terms of the average percentage for the first observation in the same experiment, the latter being considered as unity. Thus this relative percentage for the first observation is 1.00 in every experiment and the other relative values range from 1.03 (Feb. 18, Exp. 9) to 0.03 (Dec. 22, Exp. 2). To avoid possibility of confusion it may be emphasized that each value in the next to the last column of the table is the average of the individual water-

TABLE V. *Water-contents and dry weights of tops of sand-grown Norway spruce seedlings at times of successive observations in each experiment, together with corresponding cumulative values of water loss from the white atmometer sphere.*

Exp. no., etc.	Date of observation	Total corrected atmometer loss[1]	Average dry wt. per plant top.	Water content of plant tops		
				Average per top.	Ave. of water content percentages based on corresponding dry weights	
					Actual	Relative to 1st value as unity.
	(1929)	*Ml.*	*Mg.*	*Mg.*	*Per cent.*	
Exp. 1	May 27	0	7.6	29.9	396	*1.00*
24 da. period,	29	17	7.4	29.4	400	*1.01*
excessive	31	32	7.7	31.2	405	*1.02*
watering.	June 2	69	7.8	28.5	375	*0.95*
	3	85	7.8	25.0	332	**0.84**
	4	96	9.1	28.1	310	0.78
Exp. 2	Dec. 4	0	6.3	24.1	383	*1.00*
24-da. period,	6	38	6.2	24.7	385	*1.01*
standard	8	77	6.2	24.2	372	*0.97*
watering.	9	97	6.7	23.1	340	**0.89**
	10	115	6.6	22.0	323	0.84
	11	131	6.8	19.9	296	0.77
	12	145	5.7	17.0	300	0.78
	14	171	6.4	12.8	184	0.48
	18	232	6.4	5.9	92	0.24
	22	303	7.2	0.9	13	0.03
	(1930)					
Exp. 3.	Jan. 12	0	5.9	24.5	416	*1.00*
24- da. period,	14	34	5.7	23.8	414	*1.00*
standard	16	59	6.0	23.9	405	*0.97*
watering.	17	75	5.9	21.4	353	**0.85**
	18	94	5.7	18.7	324	0.78
	19	111	6.4	21.0	330	0.79
	20	133	6.3	19.0	301	0.72
	21	151	6.3	18.5	296	0.71
	22	167	6.6	17.9	283	0.68
	23	182	7.0	17.9	256	0.62
	24	202	6.6	13.7	206	0.50
	25	227	6.7	11.0	142	0.34
Exp. 4	Jan. 12	0	6.0	23.9	403	*1.00*
24- da. period,	14	34	5.8	23.4	401	*1.00*
excessive	16	59	5.9	23.0	387	*0.96*
watering.	18	94	6.3	21.8	346	**0.86**
	19	111	5.9	20.0	336	0.83
	21	151	6.6	20.0	302	0.75
	23	182	6.5	17.6	289	0.72
	25	227	6.5	10.8	206	0.51
Exp. 5.	Feb. 20	0	4.7	20.6	445	*1.00*
	22	71	5.2	21.1	374	0.84
24-da. period,	23	95	5.6	20.0	338	**0.76**
deficient	24	109	5.8	19.1	330	0.74
watering.	25	132	5.5	11.5	170	0.38
	26	165	5.7	8.9	113	0.25
	28	200	5.8	3.5	37	0.08

[1] From white sphere, since first observation.

TABLE V *(continued)*.

Exp. no., etc.	Date of observation	Total corrected atmometer loss[1]	Average dry wt. per plant top.	Water content of plant tops		
				Average per top.	Ave. of water content percentages based on corresponding dry weights	
					Actual	Relative to 1st value as unity.
	(1930)	*Ml.*	*Mg.*	*Mg.*	*Per cent.*	
Exp. 6.	Mar. 20	0	5.7	23.1	411	*1.00*
	21	28	5.2	22.0	417	*1.02*
24-da. period,	22	56	5.3	21.7	405	*0.99*
standard	23	81	5.4	21.5	400	*0.97*
watering.	24	117	5.9	18.5	327	**0.80**
	25	128	6.0	17.9	300	0.74
	26	141	6.2	16.2	285	0.69
	28	179	5.6	10.0	172	0.42
	31	238	5.7	2.8	42	0.10
Exp. 7.	Feb. 13	0	7.8	17.0	219	*1.00*
	16	52	8.4	18.2	217	*0.99*
60-da. period,	17	75	7.9	17.0	216	*0.99*
standard	18	94	7.6	14.6	190	**0.87**
watering.	20	152	8.2	8.0	99	0.45
	22	223	8.6	2.4	26	0.12
	27	330	8.1	1.6	20	0.09
Exp. 8.	Jan. 15	0	7.9	17.0	216	*1.00*
	17	30	8.3	17.7	212	*0.98*
68-da. period,	18	49	8.6	17.5	203	*0.94*
standard	19	66	8.3	16.9	206	*0.95*
watering.	20	88	8.2	16.0	202	0.94
	21	106	7.9	15.7	199	0.92
	22	122	9.0	17.0	185	**0.86**
	23	137	8.9	13.3	149	0.69
Exp. 9.	Feb. 13	0	9.9	16.1	166	*1.00*
	16	52	9.2	15.2	167	*1.01*
97-da. period,	18	94	9.7	16.5	170	*1.03*
standard	20	152	9.0	15.0	168	*1.01*
watering.	22	223	9.4	12.1	127	**0.76**
	23	247	10.5	11.3	110	0.66
	25	284	10.0	11.4	116	0.70
	27	330	9.7	11.3	118	0.71
	Mar. 1	380	9.4	6.0	62	0.37
	4	434	10.1	1.3	13	0.08

content ratios of the several seedling tops in the given sample; it is not the quotient of the average water-content per seedling top (third from last column) divided by the corresponding average dry weight (fourth from last column). For example, for May 27 in Experiment 1 the average ratio of water-content to dry weight is 396, as given, while the ratio of the average water-content (29.9 mg.) to the average dry weight (7.6 mg.) would be 393. It was thought that the method of computation used would be somewhat more

precise than the less laborious one, but the discrepancies between the results obtained by the two methods are not significant in this particular study. The average green weights are not shown in the table but each green-weight value is of course simply the sum of the corresponding dry-weight and water-content values, both of which are given.

The atmometric totals.—In the third column of Table V are presented the corrected losses from the white atmometer sphere. For each observation date in each experiment the total atmometer loss is given for the period since the day after the last watering. This last watering corresponds, of course, to the last effective rain before the onset of a drought period under natural conditions. The nature of a drought period may be defined partly in terms of its duration and partly in terms of its evaporation conditions, which generally fluctuate in intensity from day to day. But, as Wilson ('27) has pointed out, atmometric losses integrate time and evaporational intensity and the total water loss from a suitable standard evaporation surface constitutes a useful measure of the evaporational character of the period considered, including the time factor. Cumulative atmometric water-loss, as given in Table V, may serve better than mere time for picturing the march of the environmental conditions that influence water loss from the plants and from the soil through evaporation. But the time data are also given in the table.

The dry weights.—Since water contents were computed as percentages of the corresponding dry weights, it is desirable to examine the dry-weight values in some detail. A study of the nine series of these for Norway spruce shows that the average dry weight of tops increased very slightly, if at all, during the experiment period. Throughout the period the seedlings apparently lost through respiration almost as much non-water material as was gained through photosynthesis. Absorption of non-water material from the soil was surely negligible in this connection. It is to be remembered that a relatively small amount of this material was discarded, since only stems and leaves were taken in the samplings, the roots being thrown away, and a very little of the material derived from the photosynthetic process went into the root portion of the plant. In this phase of development the roots were enlarging rapidly and their enlargement may have continued to some extent after enlargement of the aerial parts had ceased because of drought.

In Experiment 1 the average dry weight shows no significant alteration throughout the 9-day period, with the single exception of the last value. All values excepting the last lie between 7.4 and 7.8 mg. That the last value, for June 4, is as high as 9.1 mg. may be due to imperfect sampling of the stand of seedlings. This was the first experiment performed and some imperfections in technique of sampling were overcome in later experiments. Neglecting this exceptional value it may be said that the average dry weight of tops of these seedlings was about 7.7 mg. throughout the first experiment.

In Experiment 2 the last dry-weight value for the 19-day period is again the highest (7.2 mg.) but the next to the last is almost like the first (6.3, 6.4 mg.) and the lowest value (5.7 mg.) is for the 9th day. It appears that these plants had an average dry weight of about 6.4 mg. throughout the period and that the fluctuations shown are of negligible significance.

In Experiment 3 there seems, on the whole, to be some evidence of a slight increase in average dry weight during the 14 days of the experiment, as from 6.0 mg. to about 6.7 mg. The fluctuations are markedly inconsistent, however; we may note, for instance, that the lowest value (5.7 mg.) occurs for the third and again for the seventh day, while the highest value occurs for the twelfth day. The average dry weight for the whole period of this experiment is 6.3 mg.

In Experiment 4 the last three values are alike (6.6, 6.5 mg.) and high, but the value for the eighth day of the 14-day period is low and like that for the fifth day (5.9 mg.), while the value for the first day is about the same (6.0 mg.). The average for the period is 6.2 mg.

In Experiment 5 there is again some evidence of an increase in dry weight during the 9 day period, but the highest value (5.8 mg.) occurs for the fifth day as well as for the ninth. The average for the period is 5.5 mg.

In Experiment 6 there seems to be no evidence of increase in dry weight and the average of all values for the 12 days is 5.7 mg., which happens to be the actual value shown for the first day and the last.

The results for Experiment 7 are similar to those of Experiment 6 in this regard, with an average dry weight of 8.1 mg. for the 15-day period. These plants had been watered for about 60 days (instead of 24) after they emerged from the soil and before the beginning of the drought period.

The plants of Experiment 8 had been watered for 68 days after emerging from the soil. The record of average dry weights shows some evidence of increase, from about 8.3 mg. (second day of the 9-day experiment period) to about 8.9 mg. (last day), but the lowest value (7.9 mg.) occurs for the seventh day as well as for the first. The average for this experiment is 8.4 mg.

For Experiment 9, with seedlings that had been watered about 97 days before the experiment began, there is no evidence of any significant increase in dry weight during the 20-day period. The first and last values are essentially alike (9.9 and 10.1 mg.) and the highest value (10.5 mg.) occurs for the eleventh day. The average for this period is 9.7 mg.

To summarize—Dry weights surely did not decrease during the experiment periods and if they increased the increment was very small, so small as to be negligible in considering the use of dry weight as the basis for computing the comparative indices of plant water content. The average dry weights per seedling for the nine experiments with Norway spruce are 7.7, 6.4, 6.3, 6.2, 5.5, 5.7, 8.1, 8.4 and 9.7 mg. The three highest averages (Ex-

periments 7-9) are for seedlings that were much more advanced in development than those of the other experiments. The non-water material probably began to increase in these Norway spruce seedlings when they had been about four or five weeks above the soil. This increase should be very slow at the start but its summation apparently became considerable in a period of two or three months. The seedlings of Experiment 1 showed a much higher dry weight of tops than did any of the other lots of 24-day seedlings. This may be related to the fact that Experiment 1 was performed in May and June, with long daylight periods and high light intensities, while all the other seedlings were grown between November and March. Omitting Experiment 1, it may be said that seedlings about 24 days old (counting from their appearance above the soil) had dry weights of about 6.0 mg., while those about 60 days old had dry weights of about 8.3 mg., and the dry weights of those about 97 days old were about 9.7 mg. The rate of increase in non-water material should of course have been increasingly accelerated as the seedlings developed, especially after the first whorl of true leaves began to appear.

The diurnal fluctuation in water content of tops of healthy seedlings.—It has been mentioned that all seedling samples were taken in the early morning, shortly before sunrise, so as to have them represent the seedling population of their respective beds at about the time of the maximal water content for the day. In this connection it will be interesting to present the results of a special series of measurements of plant moisture, made in the usual way by means of 10-plant samples from a bed of 26-day-old Norway spruce seedlings rooted in well watered sand, the samples being taken at 2-hour intervals for a 24-hour period. This special experiment was begun on the morning of March 21, 1929. The day was cloudless, without unusual meteorological influences. The average water contents per seedling are shown in Table VI.

TABLE VI. *Average water-contents of Norway spruce seedlings.*

Time of observation	Average water content per plant	
	As percentage of dry weight	Relative to highest value
Hour of day (Mar. 21)	*Per cent.*	
6	417	1.00
8	415	1.00
10	393	0.94
12	394	0.94
14	385	0.92
16	380	0.91
18	397	0.95
20	401	0.96
22	405	0.97
24	397	0.95
(Mar. 22)		
2	411	0.99
4	408	0.98
6	405	0.97

The average moisture content of these seedling tops decreased as much as 9 per cent between the sixth and the sixteenth hour, but nearly complete recuperation occurred in the next few hours. Only one experiment of this kind was carried out but the results agree with what should be expected from our knowledge of the diurnal fluctuation of water content in leaves and stems generally. This sort of fluctuation was suggested by Renner ('11) and definitely pointed out by Livingston and Brown ('12). It has been reported in several more recent papers (Lloyd, '12; Edith B. Shreve, '14; Krasnoselsky-Maximov, '17; Bachmann, '22; Knight, '22; Mina and Butovski, '23; Maximov and Krasnoselsky-Maximov, '24). It is of course due to the fact that daytime transpiration is more rapid than water absorption and conduction to the transpiring parts, whereas absorption and conduction are more rapid than transpiration in the earlier hours of the night. Thus the saturation deficit incurred during the day is made up at night.

Early-morning water contents of healthy Norway spruce seedlings, before drought effects were apparent.—In each experiment the average water content per seedling top for the first observation after watering was discontinued may be taken to represent the seedlings before any injury due to moisture deficiency began. In the series of relative water-content values for each experiment (last column of Table V) the value for the first observation is always taken as 1.00, as has been said, but it will be noticed that the highest water-content value for an experiment is not always recorded for the first observation. When a later observation gives a relative value greater than that for the first the highest value may be 1.01, 1.02 or 1.03 and it may occur for an observation as late as the third, as in Experiments 1 and 9. This fluctuation is very slight, however, and it is not to be regarded as significant under the circumstances of this study. These three higher values are consequently considered as practically equivalent to 1.00.

Of course there is no way of detecting the very first stage of injurious moisture deficiency by inspection, but it may be supposed that the seedlings were still in good health until the relative water-content value had decreased somewhat below 1.00. In accordance with the general appearance of the seedlings one may tentatively, and somewhat arbitrarily, set the lower limit of relative water-content for health at between 0.94 and 0.95. It is reasonably sure that values above 0.94 always represent seedlings in which considerable turgor was still present throughout the leaves and stems, even though incipient drying of some tissues may have begun. From these considerations, a seedling may be regarded as still in a healthy condition as long as the relative water-content of its top is between 0.95 and 1.03, inclusive. All relative values above 0.94 are printed in italics in the last column of Table V.

It is interesting to note that the relative water-content values remained above 0.94 from 1 to 8 days after watering had been discontinued. Through-

out this period it is probable that the soil was gradually drying out but that its water-supplying power was still adequate to maintain health. The drying process was apparently more rapid for some experiments than for others, however, and the conditions that influenced the rate of drying of both soil and plants are presumably represented by the respective concomitant atmometer losses. A shorter period with more intense evaporation should lower the water-supplying power of the soil and the water-content of the seedlings as much as a longer period with sufficiently less intense evaporation. Also, if the soil were drier at the beginning of one experiment than at the beginning of another, a shorter period without watering should be required in the first instance to bring about the beginning of drought effects on the plants. Finally, if the root systems of the plants extended more deeply into the soil or were otherwise more extensive or more active at the beginning of one experiment than at the beginning of another, or if the water-absorbing or water-retaining power of the plants of one experiment were greater than in the case of another experiment, then the first effects of drought would be more tardy in their appearance in the former instance. With these suggestions in mind one may examine the available data on the retention of a healthy condition after watering had ceased. The values to be examined are assembled in Table VII, and are derived from Table V.

TABLE VII. *Data for Norway spruce seedlings, on the maintenance of relative average water contents of tops above 0.94, for the period of apparent health after watering had ceased.*

Exp. No., seedling age, treatment	Length of period in days	Atmometer loss for period in Milligrams	Ave. initial water content of tops expressed as percentage of dry weight,%	Initial ave. water content of tops, *Mg.*	Initial ave. dry weight of tops, *Mg.*
No. 1, 24 da., e.w.*	7	69	396	29.9	7.6
No. 2, 24 da.....	5	77	383	24.1	6.3
No. 3, 24 da.....	5	59	416	24.5	5.9
No. 4, 24 da., e.w.*	5	59	403	23.9	6.0
No. 5, 24 da., d.w.*	1	36 (?)	445	20.6	4.7
No. 6., 24 da.....	4	81	411	23.1	5.7
No. 7, 60 da.....	5	75	219	17.0	7.8
No. 8, 68 da.....	5	66	216	17.0	7.9
No. 9, 97 da.....	8	152	166	16.1	9.9

* The abbreviation *e.w.* denotes excessive watering; *d.w.*, deficient watering. All experiments not designated by one of these abbreviations had standard watering.

For experiments 2, 3, 4, 7 and 8 high water-content of tops was maintained for 5 days after the beginning of the drought and for Experiment 6 this period of high water-content was 4 days. For Experiment 1, however, the period of high water-content was 7 days. In all of these seven instances the corresponding atmometer losses were nearly alike. Their average is 69 ml., the actual loss recorded for the 7 days of Experiment 1. The lowest of these atmometer losses is 59 ml. (Exps. 3 and 4) and the highest is 81 ml. (Exp. 6). It may therefore be said that these seven experiments agree within about plus or minus 15 per cent of their average. Without attempting any great degree of precision (which would not be warranted in such a study as this) it appears that about 69 ml. of water (say between 60 and 80 ml.) was lost from the standard white atmometer sphere between the cessation of water and the end of the period of high (healthful) water contents as here defined.

This mean atmometer loss represents an integration of both time and the intensity of the atmometric conditions and it is more satisfactory for our present purpose than are the time indices alone. The time indices for these seven experiments are 7, 5, 5, 5, 4, 5, and 5 days, as has been noted, and their average is 5.14 days. The shortest period (4 da.) is 22 per cent less than the average and the longest is 36 per cent greater than the average. A healthy condition seems to have been maintained for from 4 to 7 days after watering was discontinued.

In the single instance where watering had been only barely adequate to maintain growth (Exp. 5), the drought influence was shown after only a single day and the corresponding atmometer loss is estimated as 36 ml., half of the loss for the day. This constitutes an exceptional case, the explanation of which seems to lie in the fact that this bed had an unusually low initial moisture content. Also, the seedlings themselves may have been different from those of the other 24-day beds, because of deficient water supply throughout their growing period.

The other obviously exceptional case is that of Experiment 9, in which the tops maintained an average relative water-content above 0.94 for 8 days after watering had been discontinued, during which period the standard white atmometer sphere lost 152 ml. of water. This exceptionally high value may have been due to more extensive or more efficient root systems, or to some other plant characteristic for the seedlings of Experiment 9 were 97 days old when watering was discontinued. It is notable, however, that the seedlings of Experiments 7 and 8, which were 60 or 68 days old at the beginning of the period without watering, do not appear to have been exceptional in this way; they behaved very much like the 24-day seedlings of Experiments 1, 2, 3, 4, and 6, which had received standard or excessive watering.

The absolute water-content percentages on the day after watering was discontinued ranged between 383 and 416 for the 24-day seedlings with either

standard or excessive watering. This constitutes a fairly close agreement among these beds and indicates that healthy seedlings of this class were characterized by a water-content of tops of 399 ± 16 per cent, on the dry-weight basis. For Experiment 5, the single experiment with deficient watering, the causal relations of the unusually high percentage of 445 are difficult to surmise, though several possibilities might be suggested.

A very striking point brought out by Table VII is that the older seedlings (with standard watering) were characterized by exceptionally low water-content percentages when still in good health. While the 24-day seedlings gave an average water-content of tops of about 399 per cent, the 60-day and 68-day stands (Exps. 7 and 8) gave average water-contents of tops of only 219 and 216 per cent and the corresponding percentage for the 97-day stand (Exp. 9) was as low as 166. These observations are worthy of study, for the water-content percentage is of course a ratio (water-content divided by dry weight) and when the value of a ratio changes the change may be due to a change in the numerator, to a change in the denominator or to both acting together. To examine this interesting state of affairs it is essential to consider the average actual water-content and the average actual dry weight for the beginning of each experiment. These values are shown in the last two columns of Table VII. They correspond, in each case, to the initial water-content percentage, which is taken as unity for the relative percentages of Table V.

Omitting Experiment 5 (with deficient watering), we may average the initial actual average water contents for all cases of 24-day seedlings with either standard or excessive watering, for their deviations are not great. The result is 25.1 mg. The corresponding initial average dry weight for this 24-day class with standard or excessive watering is 6.3 mg. For the healthy 60- to 68-day seedlings with standard watering, the average water content is 17.0 mg. and the average dry weight is 7.85 mg. And for the 97-day seedlings these averages are 16.1 mg. and 9.9 mg. respectively. In the 40 days of culture between the 24-day stage of development and the 60- 68-day stage the average amount of *non-water material* per seedling top increased from about 6.3 mg. to about 7.9 mg. (or from 1.00 to 1.24) and in the 73 days ending in the 97-day stage it increased from about 6.3 to about 9.9 mg. (or from 1.00 to 1.57). For the same periods the *average water-content per top* decreased from 1.00 to about 0.68 (60—68-day class) and from 1.00 to about 0.64 (97-day class). For the 60-to-68-day seedling the water-content percentage decreased from 1.00 to about 0.55 and for the 97-day seedlings this change was from 1.00 to about 0.42. In the first instance the dry-weight increase was about 18 per cent more effective in lowering the percentage value than was the water-content decrease. In the second instance the dry-weight increase and the water-content decrease were about equally effective.

Changes in average dry weight and average water content as the seedlings developed during the period of watering.—What was said in the last portion of the preceding subsection led to the interesting conclusion that, beginning with healthy seedlings 24 days old (from the time they emerged from the soil), the average dry weight per seedling top increased and the average water content per top decreased, while watering was still being continued. More detailed data bearing on this point were secured by sampling the stands for Experiment 7, 8 and 9 at intervals in the latter part of the period of watering, before the experiments on drought effects were begun. The first sampling occurred when the seedlings were 28 days old. The numerical results are shown in Table VIII, which includes some of the values shown in Table V.

Because the data in Table VIII are of special interest with reference to the internal water relations of such seedlings as these while still in a

TABLE VIII. *March of average dry weight and average water-content of healthy Norway spruce seedlings, beginning after the plantlets emerged from the soil.*

Exp. No.	Date of observation (1930)	Age of seedlings, *Days*	Average dry weight per seedling top, *Mg.*	Average water-content per seedling top	
				Absolute, *Mg.*	On basis of dry weight, *Per cent.*
7	Jan. 12	28	5.4	21.9	407
	Jan. 14	30	5.7	22.0	389
	Jan. 19	35	5.8	20.2	348
	Jan. 28	44	6.7	18.0	269
	Feb. 13*	60	7.8	17.0	219
	Feb. 16*	63	8.4	18.2	217
	Feb. 17*	64	7.9	17.0	216
8	Dec. 6	28	5.9	24.9	419
	Dec. 10	32	6.1	22.5	372
	Dec. 19	41	6.5	21.6	334
	Dec. 25	47	7.0	20.9	302
	Jan. 11	64	8.1	17.7	221
	Jan. 13	66	7.4	17.1	232
	Jan. 15*	68	7.9	17.0	216
	Jan. 17*	70	8.3	17.7	212
	Jan. 18*	71	8.6	17.5	203
	Jan. 19*	72	8.3	16.9	206
9	Dec. 6	28	6.4	25.9	396
	Dec. 10	32	6.4	23.8	376
	Dec. 19	41	6.1	21.3	352
	Dec. 25	47	6.3	20.2	323
	Jan. 11	64	8.5	18.3	219
	Jan. 17	70	8.6	19.2	225
	Jan. 28	81	8.6	18.1	198
	Feb. 13*	97	9.9	16.1	166
	Feb. 16*	100	9.2	15.2	167
	Feb. 18*	102	9.7	16.5	170
	Feb. 20*	104	9.0	15.0	168

* Data for the dates marked with asterisks are repeated from Table V.

healthy condition, as in a nursery bed, they are shown graphically in Fig. 1. The graphs for average water-content and average dry weight per seedling top (both of which are expressed in milligrams) are all plotted on the same ordinate scale but the graphs for water-content percentage are on another scale of ordinates and each one has been shifted arbitrarily so as to make its first point coincide with the first point on the corresponding graph of average water-content. All graphs are plotted on the same scale of abscassas, which shows the dates of observation and the increasing ages of the seedling stands. These data show how the average dry weight per seedling top increased, from 28 days after appearance above the soil to the end of the series of observations, and how the average actual water-content per seedling top decreased. If these values were to continue to alter in the manner shown there should come a time when the non-water material would equal and surpass the corresponding water-content. It is also clearly shown how the water-content percentage, on the dry-weight basis, decreases with the progress of development, at a rate much more rapid than that at which the actual water-content decreases. These observations substantiate the conclusions brought forth inductively in the latter part of the preceding subsection.

The graphs of water-content of tops.—The relative values of the water-content percentage on the basis of dry weight (the last column of Table V) are shown for the nine experiments with Norway spruce by the continuous-line graphs of Figures 2 to 10, where total atmometric losses since the beginning of the drought period are plotted on the axis of abscissas and the relative values of plant water content are ordinates. The observation dates corresponding to the several atmometric values are shown on the upper margin of each figure, so that simple time relations may be read directly. Each graph shows by its slope the varying rate at which the average water content of the stems and leaves altered with respect to the progress of water loss from the standard evaporating surface of the white atmometer sphere during the period of the experiment.

Every one of these graphs starts with an ordinate of 1.00 but soon bends downward. A circle on each graph marks the points for an ordinate of 0.95, which, as has been said, may be taken to represent in each experiment about the end of the period of health. The downward slope is maintained, in general, to the end of the graph, some experiments having been continued long enough to bring the last ordinate to a value below 0.10. On each graph the ordinate value corresponding to the attainment by the plants of the critical stage of wilting is indicated by a short horizontal line across the graph. As has been said, this stage (of notable shrivelling of stem and leaves accompanied by the beginning of apparent twisting) may correspond rather closely to the onset of the phase called "permanent wilting" by Briggs and Shantz

('11) and later writers. The soil-moisture graphs, which are the broken lines in these figures, will receive attention farther on, after these plant graphs have been examined.

Water-content of tops at the critical stage of wilting.—It is important to enquire whether the attainment of the critical stage of wilting in these Norway spruce seedlings might have been detected by means of water-content measurements, which might thus have replaced the less precise and somewhat impressionistic ocular observations on wrinkling and twisting. The water-content percentages that correspond to the critical degree of wilting are shown in bold-face type in the last column of Table V and they are also indicated on Figures 2 to 10, as has just been noted. Table IX shows the absolute and relative values of this percentage for each of the nine experiments with Norway spruce seedlings. It is at once clear that the *absolute*

TABLE IX

Exp. n., seedling age, and treatment	Water-content percentage of tops, on basis of dry weight at critical stage of wilting.	
	Absolute	Relative
	Per cent.	
Exp. 1. 24 da., excessive watering....	332	0.84
Exp. 2. 24 da., standard watering....	340	0.89
Exp. 3. 24 da., standard watering....	353	0.85
Exp. 4. 24 da., excessive watering....	346	0.86
Exp. 5. 24 da., deficient watering....	338	0.76
Exp. 6. 24 da., standard watering....	327	0.80
Exp. 7. 60 da., standard watering....	190	0.87
Exp. 8. 68 da., standard watering....	185	0.86
Exp. 9. 97 da., standard watering....	127	0.76

water-content percentage is not a constant throughout the series. Somewhat like the corresponding value for healthy seedlings at the beginning of the period without watering, it appears to approach constancy for the first six experiments (24-day seedlings) and for experiments 7 and 8, but its magnitude is very different for the three age classes. For the 24-day seedlings (Experiments 1 to 6) the average water-content percentage at critical wilting is 339 with a range from 327 to 353. For the 60-68-day seedlings (Experiments 7, 8) the two available values are 190 and 185, with an average of 188. For the 97-day seedlings (Experiment 9) the only available value is 127. Although the data for the more advanced seedlings are not so numerous as is desirable, it is clearly indicated that the absolute water-content percentage at the critical stage varied with the initial state of the plants, being smaller as the plants were more advanced in development. This conclusion would be expected from what has been said concerning the corresponding absolute

values for the time when watering was discontinued, while the plants were still unaffected by water deficiency.

Turning to the *relative* values of these water-content percentages at the critical stage of wilting, the range of these for the entire series of 9 experiments is only from 0.76 (Experiments 5 and 9) to 0.89 (Experiment 2) and the distribution of the variations at once suggests that one is here dealing with an approximately constant index. The range is remarkably narrow, considering the difficulty of fixing upon the critical degree of wilting by ocular observation. For instance, there was considerable doubt in Experiment 5 whether the critical stage was really attained on February 22 or on February 23. If the earlier date had been recorded, then the critical relative-percentage value would have been 0.84 instead of 0.76. Similarly the critical value 0.76 in Experiment 9 may be somewhat too low and the value 0.89 in experiment 2 may be somewhat too high. In this connection it is to be remembered that the progress of wilting and the decrease in water-content of tops was recorded only at day intervals because the diurnal fluctuation of turgidity and seedling water-content precluded the employment of more frequent observations. No great precision could be expected from daily observations on these rapidly and continuously changing features, even if the ocular criteria for the attainment of critical wilting had been wholly satisfactory, which they were not. However, taking the figures as they came, the average relative water-content percentage for the critical wilting stage of these Norway spruce seedlings is indicated as about 0.83 ± 7. It is important to note that this critical value appears to have been approximately constant for all ages of seedlings employed and for all degrees of watering.

These results suggest that relative water-content percentage of tops might have served as a criterion for detecting a critical stage of wilting corresponding to the onset of what has been termed permanent wilting and that it might have been more satisfactory than ocular observation. For these experiments with Norway spruce seedlings we might say that this critical stage was attained when the water-content of tops, on the basis of dry weight, had been decreased by about 15 or 20 per cent of the corresponding value for the healthy plants before drought influence set in. Considering the extremely complex nature of wilting relations, this statement must be regarded as of a fair degree of precision. At any rate it is more satisfactory than any possible statement about the advent of the beginning of permanent wilting that might be based on ocular observation of wrinkling and twisting alone.

A second critical value of the water-content of tops.—Another critical moisture content of seedling tops appears on some of the graphs for Norway spruce (Figs. 2-10) and is suggested on others, in the region of ordinate values about 0.70 or 0.80. When this critical value appears it is immediately followed by a temporary retardation in the rate of decrease in water-content, which is followed in turn by the final rapid decrease that leads to desiccation.

Experiment 1 (Fig. 2) was not continued long enough to show this feature. The graph for Experiment 2 (Fig. 3) shows a retardation in the rate of water loss between ordinates 0.77 and 0.78, for December 11 and 12. The graph for Experiment 3 (Fig. 4) is similar, with a retardation between ordinates 0.78 and 0.79, for January 18 and 19. On the graph for Experiment 4 (Fig. 5) there is a suggestion of a region of slower decrease in water-content between ordinates 0.75 and 0.73, but this may not be significant. The graph for Experiment 5 (Fig. 6) shows a region of retardation immediately beyond the main critical point, between ordinates 0.76 and 0.74, for February 23 and 24. As has been suggested, the main critical value may be somewhat too low in this case. In a similar manner, the graph for Experiment 6 (Fig. 7) suggests a region of retardation in water-content decrease between ordinates 0.80 and 0.74, for March 24 and 25, immediately beyond the point for the main critical stage of wilting. The graph for Experiment 7 (Fig. 8) shows no retardation at all. The one for Experiment 8 (Fig. 9) shows no region of retardation corresponding to the others, although it shows a definite one between the two ordinates of 0.94, preceding the permanent-wilting point. This appears not to be significant. A 4-day retardation is shown on the graph for Experiment 9 (Fig. 10), between ordinates 0.66 and 0.71, for February 23 and 27.

It does not seem likely that the water-content percentage is to be taken as actually *increasing* in this region of retardation in its rate of decrease, as appears on the graphs for Experiments 2, 3 and 9 (Figs. 3, 4, 10). For the present it seems best to conclude simply that the onset of the critical stage of wilting appears generally to have been followed by a temporary slowing of the rate of water-content decrease, perhaps resulting from the breaking of the tensile water columns in the vessels. This may correspond to the temporary rise in transpiring power reported by Bakke ('15) and Nichiporovich ('26). Bakke suggests that the rise may be due to the rupture of the tensile water columns.

As far as the writer is warranted in generalizing at present, he may say that the data of Tables V and VII and the graphs of Figures 1-9 indicate the following picture of the drying out of these seedlings. For about five days after the water has been cut off the water-content of the tops shows little diminution. But after about this interval the water-content decreases rather rapidly to the onset of permanent wilting (when about 17 per cent of the original water-content has been lost). Wilting continues and when about 25 per cent of the original water-content has been lost a temporary slowing of the rate of decrease in water-content is apt to occur; after this drying proceeds rapidly to the point of desiccation. Death may perhaps occur when about 30 or 40 per cent of the original water-content is lost, but this is uncertain for the lethal point has not been carefully studied.

The Initial Water-Supplying Power of the Soil, as Related to Wilting and to the Critical Water-Content of Tops

When the critical stage of wilting had been reached, or was seen to be approaching, frequent soil-point readings on the sand bed were begun and continued for several days or longer. These were made when the seedling samples were taken, shortly before sunrise. From three to five simultaneous readings, with different soil points and different locations, were regularly made at each observation and the results were averaged. It will be remembered that the soil-point reading represents the number of milligrams of water absorbed from the soil by a single soil point in one hour and that these readings are for a soil depth of from 3 to 5 cm. They serve as indices of the initial water-supplying power of the soil at the depth in question, referring to an absorbing area of about 12 sq. cm. For example, a reading of 24 mg. is taken to indicate that the soil adjacent to the instrument was capable of supplying water to the absorbing surface at a rate such that 24 mg. was supplied in the first hour after the placing of the soil point. Since the absorbing area of the instrument is about 12 sq. cm., this means that the soil adjacent to the absorber (which had been slightly compressed through the insertion of the latter) was capable of supplying 2 mg. of water per square centimeter of absorbing surface in the first hour after capillary contact had been established. The data from these readings are shown in Table X. They are given in full because they are of interest in connection with the usefulness of the soil-point method as well as in connection with the special problem of this study. For each experiment the average value that corresponds to the critical water-content and critical wilting stage of tops is shown in boldface type.

For Experiment 1 only one series of soil-point readings was made, corresponding to what the writer is considering as the onset of permanent wilting in the seedlings. For each of the other experiments two or more series of soil-point readings were made and in most cases the critical value (last column of Table 10) is for the second or third observation. The data in the last column of Table X are shown graphically by points and broken lines in Figs. 2—10, along with the corresponding graphs of water-content of seedling tops, and the critical point is marked by a value in parentheses. The ordinate scale for the water-supplying power values is numerically the same as for the relative water-content values, but the unit for the former is 1 mg. instead of 0.01. Thus, in Fig. 2, the point for a supplying power of 59 mg. is located on the level of 0.59 as read on the scale at the left.

The data and graphs for Experiments 3, 4 and 5 (Figs. 4—6) furnish a picture of the sort of results secured by means of soil points. The soil index is seen to have increased more rapidly than the water-content of seedling tops for the period under observation. Of course this decrease in the initial water-supplying power of the soil is due to the gradual drying of the soil,

TABLE X. *Soil-point readings, showing initial water-supplying powers of the soil at the 3-5 cm. depth in beds of Norway spruce.*

Exp. No., etc.	Date of Observation	Water absorbed by single soil point in 1 hr.					
		Individual tests					Average
		a	b	c	d	e	
	(1929)	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Exp. 1.							
24 da., excessive watering	June 3	49	54	58	62	71	**59**
Exp. 2.	Dec. 8	31	34	34	38	42	36
24 da., standard watering....	9	24	25	27	27	29	**26**
	10	19	19	22	24	24	22
	11	12	15	22	24	24	19
	22	4	7	10	111	—	11
	(1930)						
Exp. 3.	Jan. 14	91	95	100	—	—	95
	16	37	39	40	—	—	39
24 da., standard watering....	17	24	26	28	28	—	**27**
	18	18	23	24	29	—	**24**
	19	15	17	20	20	—	18
	20	17	19	21	25	—	20
	21	13	14	15	—	—	14
	22	11	12	14	16	—	13
	23	10	10	10	12	—	10
	24	6	7	9	12	—	8
	25	4	4	6	8	—	6
Exp. 4.	Jan. 16	110	129	144	—	—	128
	18	49	59	60	62	—	**58**
24 da., excessive watering....	19	29	32	35	—	—	32
	21	17	18	18	—	—	18
	23	20	21	21	22	—	21
Exp. 5.	Feb. 20	102	129	162	—	—	131
	22	24	27	30	31	—	28
24 da., deficient watering....	23	20	20	21	21	—	**20**
	24	14	15	15	15	—	15
	25	12	12	13	16	—	13
	26	10	10	12	15	—	12
Exp. 6.	Mar. 20	87	97	107	113	—	101
24 da., standard watering....	24	22	25	27	29	—	**26**
Exp. 7.	Feb. 17	25	28	29	33	—	29
	18	18	18	18	26	—	**20**
60 da., standard watering....	20	10	10	11	16	—	12
	22	9	10	11	—	—	10
Exp. 8.	Jan. 17	26	30	41	—	—	32
	18	24	27	34	—	—	28
68 da., standard watering....	19	16	17	17	18	—	17
	20	13	14	14	16	—	14
	21	10	14	14	17	—	14
	22	9	12	13	15	—	**12**
Exp. 9.	Feb. 16	29	31	—	—	—	30
	18	19	19	25	—	—	21
97 da., standard watering....	20	17	20	22	—	—	20
	22	16	17	17	19	—	**17**
	23	11	11	12	13	—	12

under the influence of direct evaporation and of water absorption by the seedling roots.

One of the main purposes of the present study was to gain information concerning the relations between the decrease in the value of the initial water-supplying power of the soil and the onset of a critical stage of wilting, as a drought period continued. With this in mind one may now examine the soil-point values corresponding to the critical degree of wilting. These are the values shown in boldface type in the last column of Table X and specially marked in parentheses in Figs. 2—10. They are assembled below, arranged according to the conditions of the experiments from which they were derived.

For 24-day seedlings with standard watering: 26 mg. (Exp. 2), 27 mg. (Exp. 3), 26 mg. (Exp. 6).

For 24-day seedlings with deficient watering: 20 mg. (Exp. 5).

For 24-day seedlings with excessive watering: 59 mg. (Exp. 1), 58 mg. (Exp. 4).

For 60- to 68-day seedlings with standard watering: 20 mg. (Exp. 7), 12 mg. (Exp. 8).

For 97-day seedlings with standard watering: .17 mg. (Exp. 9).

It appears that the initial water-supplying power of these Norway spruce beds at the 3—5 cm. depth, at the critical stage of wilting in the plants, was not the same for all experiments in this series. It differed according to the previous treatment of the seedlings and according to their age or stage of development. This might be expected from the general consideration that plant and soil and air constitute a single dynamic system, the environment and the plant operating together, as has been emphasized by Livingston ('29). The 24-day seedlings with standard watering had roots whose absorbing surfaces were mostly within the soil-depth range referred to by the soil-point readings and the three cultures of this group were probably very nearly alike in all essentials. For these the critical water-supplying power may be taken as 26 mg. It has been mentioned that the critical wilting stage in Experiment 5 may have occurred on February 22 instead of February 23, and if that were true the critical soil value for that experiment would be 28 mg. instead of 20 mg., which would bring the seedlings with deficient watering into the same class with those that received standard treatment. The writer's information on the relations of deficient watering does not warrant further special consideration of this single case. The 24-day seedlings with excessive watering (Exps. 1 and 4) do require special consideration, however, for these are two cases which agree in having the same very high index of water-supplying power (58, 59 mg.) at the advent of critical wilting. The excessive-watering treatment had retarded root development in these two beds (Table II) and may also have lowered the capacity of the roots to absorb water. The absorb-

ing surfaces of the roots of these excessively watered seedlings were nearer the soil surface than was true for the 24-day seedlings with standard watering. Whatever may be the reasons, the excessively watered seedlings passed into the critical wilting stage when the sand of their beds at the depth tested still showed an initial water-supplying capacity more than twice as great as that shown by the same kind of soil at the same depth when seedlings that had received standard treatment attained the critical wilting stage.

For the older seedlings (Exps. 7, 8 and 9) the critical soil-point index is seen to be remarkably low (12, 17, 20 mg.). These seedlings held their water more tenaciously than did the 24-day seedlings and they wilted more gradually. It may well be that their longer root systems were drawing much of their water supply from soil depths greater than that of the soil-point readings. These roots were observed to be more branched and longer than those of the younger seedlings, a fact which supports this suggestion. Several other possible explanations might be mentioned, perhaps involving differences in the water-conducting systems of the plants of the two age classes. No differentiation based on these soil-point readings can be made between the two classes of older seedlings, however.

RESULTS WITH NORWAY PINE

General Appearance During Wilting

Experiments 10—14 were with Norway pine. The results of these will now be presented and discussed, principally from the standpoint of how they differed from or in what respects they were similar to the corresponding Norway spruce values.

The general appearance of the Norway pine seedlings as they became seriously wilted and withered did not differ greatly from that of the Norway spruce seedlings in a corresponding state. The first sign of wilting in Norway pine was that the cotyledons became duller in color. This was followed by wrinkling, barely discernible at first but gradually becoming so severe that the tips of the cotyledons were very much twisted. As desiccation progressed the seedlings ultimately became brittle and would crumble when rolled between the fingers. The critical stage of wilting was considered as having been attained, as in the case of Norway spruce, with the development of pronounced epidermal wrinkling, just at the beginning of twisting. At this stage the seedlings recovered if the bed was watered. The lethal stage occurred after twisting began but before brittleness developed.

Decreasing Water-Content in Norway Pine Seedlings as Drying Went on

Data of moisture content of tops and atmometer losses.—In Table XI are given the data on moisture content of tops for the experiments with sand

TABLE XI. *Water-contents and dry weights of tops of sand-grown Norway pine seedlings at times of successive observations in each experiment, together with corresponding cumulative values of water loss from the white atmometer sphere.*

Exp. no., etc.	Date of observation, (1929)	Total corrected atmometer loss,[1] *Ml.*	Average dry wt. per plant top, *Mg.*	Water content of plant tops		
				Average per top, *Mg.*	Ave. of percentage water: contents based on corresponding dry weights	
					Actual *Per cent.*	Relative to 1st value as unity.
No. 10.	Dec. 6	0	4.9	21.5	442	*1.00*
	8	39	4.9	20.3	419	*0.95*
24-day period,	10	77	5.0	22.5	451	*1.02*
standard	11	93	5.1	21.9	428	*0.97*
watering.	12	107	4.7	20.3	431	*0.97*
	13	119	5.0	20.6	414	0.94
	14	133	4.8	18.4	383	**0.87**
	15	150	5.2	19.2	367	0.83
	16	169	4.9	16.6	334	0.76
	18	198	5.2	15.8	308	0.70
	22	265	4.8	8.3	145	0.33
	(1930)					
No. 11.	Jan. 15	0	4.5	24.4	542	*1.00*
	17	30	4.6	23.9	521	*0.96*
24-day period,	19	66	4.9	24.1	497	0.92
standard	21	106	4.8	22.9	472	0.87
watering.	23	137	5.6	24.5	436	**0.80**
	24	157	5.8	22.6	390	0.72
	25	182	5.9	21.7	372	0.69
	28	239	5.7	20.8	365	0.68
	31	289	5.7	18.2	329	0.61
	Feb. 4	375	6.0	10.8	174	0.32
No. 12.	Jan. 15	0	4.5	22.9	510	*1.00*
	17	30	4.9	24.2	499	*0.98*
24-day period,	19	66	4.6	22.3	481	0.94
standard	20	88	5.0	23.2	469	0.92
watering.	21	106	5.4	24.9	467	0.92
	23	137	5.0	22.3	443	0.87
	24	157	5.7	22.8	400	**0.78**
	25	182	5.6	21.8	386	0.76
	28	239	5.8	21.9	376	0.74
	31	289	5.7	21.0	366	0.72
	Feb. 4	375	6.4	15.3	237	0.46
No. 13.	Feb. 23	0	4.3	23.0	551	*1.00*
	25	37	4.7	23.8	512	0.93
24-day period,	26	70	4.8	23.0	491	0.89
deficient	27	83	4.9	24.0	492	0.89
watering.	28	105	4.2	24.1	436	**0.79**
	Mar. 1	133	5.2	20.8	397	0.72
	4	187	5.6	16.5	266	0.48
No. 14.	Feb. 13	0	6.1	20.2	336	*1.00*
	16	52	6.6	19.9	306	0.91
55-day period,	18	94	6.6	20.1	295	0.88
standard	22	223	7.3	18.1	259	0.77
watering.	23	247	6.5	17.5	251	0.75
	24	261	7.3	16.7	236	**0.70**
	25	284	7.0	12.8	192	0.57
	26	317	7.3	11.1	148	0.44
	28	352	6.9	4.8	68	0.20

[1] From white sphere, since first observation.

grown Norway pine seedlings, along with the corresponding records of water loss from the white atmometer sphere. Computations were performed just as in the experiments with the Norway spruce and Table XI is arranged like Table V.

The dry weights of Norway pine seedlings.—While the Norway spruce seedlings generally showed no significant increase in average dry weight per seedling top during the period of the experiments (*i. e.,* after watering of the beds had been discontinued), the Norway pine seedlings showed a significant increase in four of the five experiments. Experiment 10 is the exceptional one; for a span of 17 days the average dry-weight per top varies only between 4.7 and 5.2 mg.

Experiments 11, 12 and 13 all show a considerable increase in average dry weight throughout the experiment period. The average dry weight for the first day of experiment 11 is 4.5 mg. and for the last day it is 6.0 mg., or 33 per cent greater. The rise was remarkably gradual in this case; the average for each two successive values is regularly greater than that for the preceding pair. Experiments 12 and 13 are similar to Experiment 11 in this respect. The actual increase amounts to 42 per cent in the case of Experiment 12. In spite of the fact that the drying period was much shorter in Experiment 13 than in Experiments 11 and 12, there was still an increase of 30 per cent in average dry weight in Experiment 13. Every value in the series is greater than the preceding one. In Experiment 14 the dry-weight increase is not so apparent because the last value is exceptionally low. Neglecting that value, however, there is an increase from 6.1 to 7.3 mg., or 20 per cent.

It appears that the Norway pine seedlings used in these experiments were generally in a phase of development where increase in non-water content had recently begun and that this change continued throughout the drought period, to the end of the experiment. Dry weight decreases during the germination of the seed and the early phases of seedling development. This is true for green plants in general. Eventually there comes a time, after the plantlet has emerged from the soil, when the photosynthetic process more than makes up for respiratory loss, at which time the non-water content of the seedling begins to increase. Whether the dry weight decreases, increases, or is maintained for a number of days depends upon how advanced the organism is and upon light and carbon-dioxide conditions. In this respect it seems that the Norway spruce seedlings of this study were generally not so far advanced as those of Norway pine. It is interesting to note, however, that the Norway pine seedlings generally had somewhat lower dry weights than were shown by Norway spruce seedlings of similar culture and of about the same age. This is not to be regarded as a generalization, for some set of culture conditions sufficiently different from those employed in this study might establish quite different relations between these species. Another observation that may be interesting is that the lowest average dry weights for Norway pine seedlings

were for those that had been grown with deficient or barely adequate water supply (Exp. 13), which is also true of the experiments with Norway spruce, as has been mentioned.

Reverting to the general increase in average dry weight of the Norway pine seedlings during the period without watering, it is remarkable that this increase continued long after the plants had attained what is here termed the critical stage of wilting. In Experiments 11, 12 and 13 there seems to be no doubt that average dry weight was still increasing between the next to the last observation and the last. At the time of critical wilting these plants had average dry weights of 5.6, 5.7 and 4.2 mg., and in the succeeding 12 or 15 days these values increased to 6.0, 6.4 and 5.6 mg., respectively. Before the last observation these seedlings were apparently dead; they had surely passed the stage where they might recover if the bed were watered, and yet the dry weight of their tops continued to increase. This relation would make an interesting subject for special study. At any rate it is worthy of remark in passing.

Early-morning water contents of healthy Norway pine seedlings, before drought effects were apparent.—The regular decrease in average water content of seedling tops began much more promptly in the experiments with Norway pine than in the experiments with Norway spruce. The first relative average water-content percentage was again taken as 1.00 in Experiments 10—14 and it is remarkable that in only one instance (Dec. 10, Exp. 10) did any subsequent relative value have a magnitude as great as unity. As in Table V, so also in Table XI (last column), all relative percentage values above 0.94 are shown in italics, to set off that portion of each series that may be considered as representing seedlings that were still in health. That period is seen to have been 12 days in Experiment 10, but it was only 3 days in Experiments 11 and 12 and only 1 day in Experiments 13 and 14. In general, it was much shorter with Norway pine than with Norway spruce.

For Experiment 10, in which the average dry weight of tops showed no clear evidence of increase during the period without watering, the decrease in average water-content percentage is explained by the corresponding decrease in average water-content per seedling top. The percentage, being a ratio value, becomes smaller as its numerator (average water content per top) is smaller, and its denominator varies only irregularly in this instance. Therefore the water-content percentages and their relative values follow the decrease in average water-content per top, as in the case of the experiments with Norway spruce.

For Experiments 11—14 water-content percentages on the dry-weight basis decreased throughout the experiment period partly because of lowered average water content per top and partly because of increased dry weight, in a manner similar to that shown by the Norway spruce seedlings that were kept watered for 60, 68 or 97 days after they appeared above the soil. Consequently

the water-content percentage and its relative value decrease for Experiments 11—14 more rapidly than the average water content decreases and also more rapidly than the average dry weight increases. It may be noted that the influence of increasing average dry weight on the magnitude of the percentage ratio is generally greater than is the influence of decreasing average water content per top. Further analysis of this interesting relation may be made from the data given in Table XI if and when such analysis is desired.

The processes that resulted in lowered water-content percentages were occurring even before the water was cut off. It cannot, therefore, be said that the drying out of the soil and the consequent decrease of its water-supplying power were wholly responsible for decrease in water content of the seedlings but merely that they accelerated to a pathological extent a series of changes that were in progress before the soil environment became unfavorable to health.

Turning to the water-content percentages that are taken as having the relative value of 1.00 in these Norway pine experiments (the first value for each experiment), it is interesting to compare their magnitudes with the magnitudes of the corresponding percentages for Norway spruce. These values are assembled in Table XII.

TABLE XII. *Comparison of water percentages of seedlings.*

Age of seedlings and culture treatment	Average water content; percentage of dry weight	
	Norway spruce, %	Norway pine
	Per cent.	*Per cent.*
24 day; standard watering.....	383, 416, 411	442, 542, 510
24 day; deficient watering.....	445	551
55 day; standard watering.....	...	336
60–68 day; standard watering.....	219, 216	...

The Norway pine graphs of water-content of tops and their critical values.—Figs. 11—15 present graphs of the relative values of water-content percentages for the five Norway pine experiments, arranged like the graphs for Norway spruce in Figs. 2—10. Soil-point data are not shown in these figures for Norway pine. The average water-content percentages that correspond to the critical stage of wilting in these experiments with Norway pine are shown below, with the corresponding critical values from the Norway spruce experiments.

It may be seen that the critical relative percentages for the 24-day seedlings with standard or deficient watering have a range from 0.78 to 0.87 for Norway pine, almost exactly the same as for Norway spruce (0.80 to 0.89). For this class of seedlings, for both species, critical wilting occurred when

the water-content percentage had decreased to about 80 per cent (pine) or 85 per cent (spruce) of its value when watering was discontinued. For the 55-day pine seedlings the critical relative water-content value appears to have been significantly lower (0.70) than the corresponding value for the 24-day seedlings. This is not true for the 60- to 68-day spruce seedlings, for which the critical values are about the same as those for the corresponding 24-day seedlings.

TABLE XIII. *Critical water-content percentages of seedlings.*

Age of seedlings and culture treatment	Critical ave. water-content percentage on basis of dry wt.			
	Norway spruce		Norway pine	
	Absolute	Relative	Absolute	Relative
	Per cent.		*Per cent.*	
24 da., standard watering ...	340	0.89	383	0.87
	353	0.85	436	0.80
	327	0.80	400	0.78
24 da., deficient watering....	338	0.86	436	0.79
55 da., standard watering ...	...	....	236	0.70
60-68 da., standard watering.	190	0.87	...	....
	185	0.86	...	....

The graphs for Experiments 10, 13 and 14 (Figs. 11, 14, 15) each slope downward regularly beyond the point representing the critical water-content percentage and no significant change in the slope of the graph is shown. For Experiments 11 and 12 (Figs. 12, 13), however, the slope of each graph decreases after the critical point has been passed. For a considerable period after the critical degree of wilting had occurred these pine seedlings maintained nearly the same water-content percentage. Beyond this gently sloping portion of the graph the slope is accelerated and again takes a direction about like the slope of the portion at the left of the critical point. It may be that the above-mentioned temporary retardation in the decrease of the water-content percentage of tops represents for Norway pine the phenomenon that was brought out by several of the graphs for Norway spruce for about the same regions. Whatever may be the explanation of the period of nearly maintained water-content percentage in these two experiments with pine, it is evident in only these two.

CRITICAL INITIAL WATER-SUPPLYING POWER OF THE SOIL FOR THE NORWAY PINE BEDS

The soil-point readings taken at the advent of the critical wilting stage in the Norway pine seedlings gave results in every way consistent with those obtained in the experiments on Norway spruce. These critical indices of the

initial water-supplying power of the soil are shown in Table XIV. Each value represents the average of four or five soil-point readings. The corresponding values for Norway spruce are also shown.

TABLE XIV. *Critical indices of water supplying power of soil.*

Age of seedlings and culture treatment	Initial water-supplying power of soil at occurrence of the critical stage of wilting.	
	Norway spruce	Norway pine
	Mg.	*Mg.*
24 da., standard watering.....	26	18
	27	20
	26	19
24 da., deficient watering.....	20	12
24 da., excessive watering.....	59	—
	58	—
55 da., standard watering.....	—	11
60 to 68 da., standard watering. ..	20	—
	12	—

As with Norway spruce, the onset of the critical stage of wilting in the pine seedlings occurred with different values of this soil index, according to the soil moisture conditions under which the plants had grown and according to the age of the plants. For the 24-day seedlings the critical soil-moisture index is markedly lower (about 19 mg.) for the Norway pine seedlings than for the Norway spruce seedlings (about 27 mg.), and this relation holds for the other groups. For both species the critical soil-moisture index is lower for the seedlings that had had deficient water supply than for those of the same age with standard watering. Also, for both species the critical index is lower for the older seedlings with standard watering than for the 24-day cultures with like treatment. For both species the three experiments with 24-day beds watered in the regular way gave remarkably similar soil-point readings for the critical water-supplying power. This observation furnishes an illustration of the highly consistent results that may be secured by means of soil points in a study of this sort.

RESULTS WITH ADDITIONAL SPECIES

General remarks.—Besides the 14 experiments for which results have been presented above, one regular experiment was carried out with seedlings of each of the following additional species: white spruce, northern white cedar, shortleaf pine, slash pine, western yellow pine, western white pine, Douglas fir, lowland white fir, western red cedar, and western hemlock. The numerical results of these ten experiments are presented in Table XV, which is in the same style as Tables V and XI.

TABLE XV. *Water-contents and dry weights of tops of sand-grown seedlings of ten species of coniferous trees at times of successive observations in each experiment, together with corresponding cumulative values of water loss from the white atmometer sphere. (In every case the seedlings were about 24 days old and had had standard watering.)*

Exp. no. and species	Date of observation 1929-1930	Total corrected atmometer loss,[1] *Ml.*	Average dry wt. per plant top, *Mg.*	Water content of plant tops		
				Average per top, *Mg.*	Ave. of water-content percentages based on corresponding dry weights	
					Actual *Per cent.*	Relative to 1st value as unity.
Exp. 15.	Dec. 10	0	3.5	15.1	427	*1.00*
	12	30	3.3	14.4	430	*1.01*
White spruce.	14	56	3.5	14.4	418	*0.98*
	15	73	3.4	13.6	395	0.92
	16	92	3.6	13.1	364	**0.85**
	17	107	3.6	11.8	330	0.77
	18	121	3.6	9.6	264	0.62
	19	131	3.5	4.8	139	0.33
	21	166	3.4	3.2	094	0.22
Exp. 16.	Dec. 12	0	1.6	6.0	388	*1.00*
	15	43	1.7	6.6	402	*1.04*
Northern white cedar.	16	62	1.6	6.6	399	*1.03*
	17	77	1.5	6.4	402	*1.04*
	18	91	1.6	5.4	322	**0.83**
	19	101	1.4	4.5	308	0.79
	20	113	1.4	3.5	249	0.64
Exp. 17.	Dec. 9	0	6.4	34.5	514	*1.00*
	11	34	6.5	32.7	514	*1.00*
Shortleaf pine.	13	60	7.0	31.6	479	0.93
	14	74	6.7	32.0	476	0.93
	15	91	8.1	33.1	486	0.95
	16	110	6.2	30.9	487	0.95
	17	125	7.6	35.4	469	0.91
	18	139	7.8	30.0	399	**0.78**
Exp. 18.	Dec. 10	0	16.9	89.8	537	*1.00*
	12	30	14.6	82.0	542	*1.01*
Slash pine.	14	56	16.6	85.9	529	*0.99*
	15	73	17.8	91.8	516	*0.96*
	16	92	15.9	72.4	516	*0.96*
	17	107	15.0	78.2	526	*0.98*
	18	121	12.9	39.9	318	**0.59**
Exp. 19.	Dec. 12	0	17.7	92.0	528	*1.00*
	17	77	17.6	92.3	528	*1.00*
Western yellow pine.	18	91	16.7	87.2	524	*0.99*
	19	101	17.8	68.0	412	**0.79**
	21	136	18.8	49.7	267	0.51
	(1930)					
Exp. 20.	Feb. 13	0	9.0	41.4	457	*1.00*
	16	52	9.9	42.1	425	0.93
Western white pine.	18	94	10.5	40.9	391	0.86
	20	152	11.1	39.8	359	0.79
	21	188	12.6	37.3	293	**0.64**

[1] From white sphere, since first observation.

TABLE XV *(continued)*.

Exp. no., and species	Date of observation 1929–1930	Total corrected atmometer loss[1]	Average dry wt. per plant top.	Water content of plant tops		
				Average per top.	Ave. of water-content percentages based on corresponding dry weights	
					Actual	Relative to 1st value as unity.
		Ml.	*Mg.*	*Mg.*	*Per cent.*	
	(1929)					
Exp. 21.	Dec. 15	0	6.4	36.8	574	*1.00*
	17	34	6.5	37.1	567	*0.99*
Douglas fir (Pacific).	19	58	6.9	37.3	556	*0.97*
	20	70	6.2	37.4	603	*1.05*
	21	93	7.6	36.1	476	**0.83**
	22	115	7.6	35.1	466	0.81
	(1930)					
Exp. 22.	Mar. 1	0	11.9	69.3	594	*1.00*
	4	54	12.3	68.3	578	*0.97*
Lowland white fir.	6	100	11.9	61.0	654	*0.95*
	7	126	12.4	54.3	448	**0.75**
	10	207	14.1	42.4	307	0.52
	13	260	12.9	41.7	322	0.54
Exp. 23.	Feb. 20	0	1.4	7.2	508	*1.00*
	22	71	1.4	6.4	466	0.92
Western red cedar.	23	95	1.4	6.1	418	0.82
	24	109	1.6	6.1	405	0.80
	25	132	1.8	4.1	140	**0.28**
	26	165	1.9	0.5	026	0.05
	28	200	1.6	0.1	010	0.02
Exp. 24.	Mar. 7	0	1.0	5.1	531	*1.00*
	9	56	1.0	5.0	519	0.98
Western hemlock.	10	81	1.0	4.8	512	0.96
	11	105	1.0	2.6	256	**0.48**
	13	134	1.0	1.9	187	0.35

In all these experiments the beds received standard watering, which was discontinued about 24 days after the plants emerged from the soil. Because of scanty germination, however, some of these experiments were carried out with seedlings of somewhat more varied age than was regular and samples of between five and six seedlings each, instead of the regular ten, were used. The experiments with white spruce, northern white cedar, shortleaf pine, and western hemlock (Exps. 15, 16, 17 and 24) were quite regular in all ways but the results of the others here considered are perhaps not quite so reliable in some respects as are those with the species just mentioned. Since there is but a single experiment for each of these ten additional species, however, the results must be regarded as no more than indicative or suggestive at best.

Decreasing Water-Content of Tops as Drying Went On

Appearance of seedlings of the ten supplementary species at the stage of critical wilting.—The critical stage of seedling wilting for each of the 10 supplementary forms with which only single experiments were performed was considered as attained when the plantlets had become clearly injured by inadequate water supply but before the cotyledons had become brittle through drying. At this stage the morphological characteristics in the case of white spruce, shortleaf pine, western yellow pine, western white pine, and Douglas fir were very similar to those for Norway spruce and Norway pine with about the same degree of drought injury. Seedlings of lowland white fir showed at this stage wrinkling and the beginning of twisting in the cotyledons and the upper region of the hypocotyl was somewhat bent as if nutation were occurring or drooping had set in. As slash pine seedlings came to the critical stage their cotyledons exhibited marked drooping, hanging obliquely downward within about 30° of the still vertical hypocotyl. Critically wilted seedlings of northern white cedar, red cedar, and western hemlock had markedly wrinkled cotyledons and wilting advanced with great rapidity in these cases; these seedlings showed no visible effects of drought on the morning of the day when the critical stage was to be attained and wilting had definitely reached the critical stage by nightfall. In the case of the two cedars, when this wilting stage was attained the middle part of each comparatively broad cotyledon had become definitely thinner than the marginal portion, through partial collapse of the underlying tissues. Thus these cotyledons showed raised margins and depressed middle regions when viewed either from above or from below. As has been said, it is practically impossible to detect by simple ocular observation the attainment of any definite stage of wilting in the seedling conifers with which this study deals and these notes on the appearance of critically wilted plantlets are consequently much less definite than would be desirable if more satisfactory descriptions were possible.

The dry weights.—White spruce, northern white cedar, slash pine, western yellow pine and western hemlock resembled Norway spruce in showing no significant increase in the dry weight of seedling tops within the experiment period. The dry weights of the seedlings of shortleaf pine, lowland white fir, and western red cedar may have increased slightly. The experiments with western white pine and Douglas fir showed a steady and unquestionable increase in dry weight. For western white pine the final average dry weight is 40 per cent higher than the initial one and for Douglas fir an average dry-weight increment of 19 per cent is shown.

This question of increase in average dry weight per seedling is important in connection with the interpretation of the decrease in water-content percentage, as has been pointed out. It also furnishes one kind of dynamic criterion by which the physiological maturity of a stand of seedlings may be

estimated. Those seedlings that showed no increase in dry weight during the experiment period would have shown increase if they had been somewhat more advanced in development and those that did show definite increase in dry weight would have shown a higher rate of increase if watering had been maintained somewhat longer.

Early-morning water-contents of healthy seedlings, before drought effects were apparent.—The seedlings of most of the species here considered followered the course of the Norway spruce seedlings in exhibiting a considerable period, after the water supply had been cut off, during which the relative water-content of tops remained above 0.94. For white spruce this period lasted for 5 days (the average duration for Norway spruce), for northern white cedar it continued for 6 days and for shortleaf pine 8 days elapsed during which there was no significant decrease in the water-content percentage, although it is true in this instance that two intermediate values were as low as 0.93. The slash pine seedlings also maintained an average water-content percentage above 0.94 for 8 days; those of western yellow pine, for 7 days; and those of Douglas fir and lowland white fir, for 6 days. The seedlings of western hemlock, which had very shallow root systems, were able to maintain apparent health for 4 days after watering was discontinued. These time periods may be translated into terms of atmometer loss by means of the data given in Table XV. The seedlings of western white pine and western red cedar showed relative average water-content percentages below 0.94 at the time of the second observation and the decrease in this index value was scarcely accelerated at the onset of the critical stage of wilting.

Water-contents of tops at the critical stage of wilting.—The actual and relative water-content percentages of tops (on basis of dry weight) that corresponded to the occurrence of the critical stage are shown in Table XVI.

The unusually low water-contents for western red cedar and western hemlock (Exps. 23 and 24) may have been due to the very limited extent to

TABLE XVI. *Actual and relative water-contents of various conifer seedlings.*

Exp. No.	Species	Critical water-content percentage of tops on basis of dry weight	
		Absolute	Relative to 1st value in series
		Per cent.	
15	White spruce	364	0.85
16	Northern white cedar	322	0.83
17	Shortleaf pine	399	0.78
18	Slash pine	318	0.59
19	Western yellow pine	412	0.79
20	Western white pine	293	0.64
21	Douglas fir	476	0.83
22	Lowland white fir	448	0.75
23	Western red cedar	140	0.28
24	Western hemlock	256	0.48

which the root systems of these seedlings had developed at the time observations began. These had by far the shallowest roots of any of the forms tested. Furthermore, these two experiments were carried out at times of unusually intense evaporation, and drying out proceeded with unusual rapidity. The low water-content percentage for western white pine (Exp. 20) at the occurrence of the critical wilting stage may have been due to inadequate sampling of the stand. In any event, this was the most inadequately sampled of any of the species tested. Of the remaining seven experiments in this group, all but Experiment 18 (slash pine) show critical water-content percentages within the same general range as was shown for Norway spruce and Norway pine.

Critical Initial Water-Supplying Powers of the Soil for the Supplementary Experiments

The average soil-point readings corresponding to the critical stage of wilting for the ten supplementary species are shown in Table XVII. The readings from the 24-day experiments with standard watering for Norway spruce and Norway pine are also included in the list, each of these values being the average from three experiments. The values are arranged in the order of increasing magnitude.

Table. XVII. *Critical soil point readings.*

Exp. No.	Species	Critical soil-point reading
		Mg.
19	Western yellow pine	11
17	Shortleaf pine	12
18	Slash pine	13
21	Douglas fir	14
22	Lowland white fir	14
23	Western red cedar	14
10, 11, 12	Norway pine	19
20	Western white pine	20
16	Northern white cedar	22
24	Western hemlock	25
2, 3, 6	Norway spruce	26
15	White spruce	30

The twelve species appear to fall into three groups according to these critical soil-point values: (1) those with critical values from 11 to 14, (2) those with values from 19 to 22 and (3) those with values from 25 to 30. This classification may be suggestive in connection with the relative drought resistance of seedlings of the several species, but it is based on single experiments only, excepting for Norway spruce and Norway pine, and in any event this arrangement naturally refers only to the lots of seed used in this study, to the general technique employed in the writer's experiments, and to the climatic conditions of these experiments.

SUMMARY AND CONCLUSIONS

The studies here reported were made in the Laboratory of Plant Physiology of the Johns Hopkins University during 1929 and 1930. Sand beds of seedling coniferous trees of 12 species were maintained with good moisture conditions in a greenhouse room for about 24 days after the plantlets had emerged from the soil, and for longer periods in some instances. Most of the beds were watered in the ordinary way (standard watering) but in a few instances watering was either excessive or deficient. Deficient watering implies a degree of watering just adequate to maintain health. The beds with excessive watering were kept considerably wetter than those with standard watering. After the growing period the cultures were allowed to dry out without further watering and the progress of wilting and drying was followed by means of frequent ocular observations on the plants as well as by means of average water-content measurements made from day to day on the tops of representative 10-plant samples, the roots of the sample seedlings being discarded. The water-content values were expressed as percentages on the basis of dry weight and these percentages for each experiment were also expressed as a series of relative values, considering as 1.00 the average percentage given by the first sample of tops, taken on the day following the cessation of watering. The average dry weight per seedling increased during the period of drying out for some species and remained without significant change for other species. Data on average dry weight per seedling and on average actual water-content per seedling are presented and discussed, as well as the moisture-content percentages.

Nine experiments were performed with seedlings of Norway spruce (*Picea excelsa* Karst.) and five experiments were on seedlings of Norway pine (*Pinus resinosa* Ait.). Only a single experiment was performed with each of the following forms: white spruce (*Picea glauca* (Moench) Voss.), northern white cedar (*Thuja occidentalis* L.), western red cedar (*T. plicata* D. Don.), shortleaf pine (*Pinus echinata* Mill.), slash pine (*P. caribaea* Morelet), western yellow pine (*P. ponderosa* Laws), western white pine (*P. monticola* D. Don.), Douglas fir (*Pseudotsuga taxifolia* Britt.), lowland white fir (*Abies grandis* Lindl.) and western hemlock (*Tsuga heterophylla* Sarg.).

Two observational stages of wilting and drying of the leaves and stems were recognized: a critical stage of wilting, probably corresponding to the onset of what has been called permanent wilting, and a stage of withering, at which the leaves and stems became brittle. Permanent wilting is taken to mean that phase of wilting in which the seedlings could not recover if transpiration were stopped but could recover promptly if the soil were adequately watered. The critical stage of wilting, at which the plants were still alive, was marked by considerable wrinkling of hypocotyl and cotlyedons and the beginning of a characteristic twisting of the cotyledon tips.

The diurnal fluctuation in plant moisture content, which has been described for other plants by several experimenters, was specially studied for Norway spruce. Because of this fluctuation all samples for measurement of the water contents of seedling tops were taken just before sunrise, before the daytime lowering of water content had set in.

When the first critical stage of wilting (approximately the beginning of the phase of permanent wilting) was seen to be approaching, or when that stage was judged to have been attained, measurements of soil-moisture conditions were made and in a number of experiments these were repeated at frequent intervals until the second critical stage of wilting (brittleness of leaves) had been reached. Soil-moisture measurements were made by means of standardized Livingston soil points, several simultaneous readings being taken, with different instruments, at each observation. The results show how the initial water-supplying power of the soil decreased as the beds dried out and the critical wilting stage was approached and passed. These soil-point readings all refer to an exposure of the instrument for one hour at a depth of 3—5 cm. below the surface of the bed. They are given in terms of milligrams of absorption per soil point and refer to an absorption area of about 12 sq. cm. A special series of tests showed the relation between soil-point reading and soil-moisture content for the upper 4 cm. of soil in a bed like those used for the seedlings. By means of a table based on the results of these tests the supplying-power values may be approximately translated into water-content values.

Records were kept of the climatic conditions of the greenhouse room in which the experiments were performed and the duration factor for the experiments was expressed in terms of total water loss from a standardized Livingston porous-porcelain atmometer sphere as well as in terms of time. Each observation of plant moisture and of soil moisture is shown as having occurred when the white atmometer sphere had lost a specified amount of water since the day following the cessation of watering of the seedling bed. But the corresponding number of days is also given in every case. The atmometric measure is an integration of time and the varying intensity of evaporation conditions and it is consequently more satisfactory than time alone in studies of this sort, when climatic conditions fluctuate in any experiment period.

It was found that the critical stage of wilting, as judged by ocular observation, corresponded fairly closely to a certain amount of decrease in the percentage representing the water-content of tops. Thus the average water-content percentage (on the basis of the corresponding dry weight of tops) furnished a more satisfactory criterion for detecting the onset of the critical stage of wilting than could be had by ocular observation. For the experiments with Norway spruce and Norway pine seedlings the attainment of the critical stage of wilting generally occurred when the average water-content

percentage had decreased by about 15 or 20 per cent of the corresponding value for the healthy plants before drought influence set in. The single experiments with the other 10 forms suggest that this critical percentage may be expected to vary according to species, but this is no more than a suggestion. It is also suggested that the soil-moisture conditions that prevailed during the growth period may have exerted some influence on the extent to which the water-content percentage had decreased at the onset of the critical stage of wilting. Considering the water-content percentage for healthy seedlings as 1.00, the corresponding percentage for the attainment of critical wilting ranged between 0.76 and 0.89 for all experiments with Norway spruce seedlings, between 0.70 and 0.87 for all experiments with Norway pine seedlings and between 0.28 (western red cedar) and 0.85 (white spruce) for the ten single experiments with other species.

Experiments with seedlings more than 24 days old (since emerging from the soil) indicated that the average dry weight per seedling top in health increased with advancing development while the corresponding average water-content per top decreased. The water-content percentage for healthy seedlings therefore decreased markedly as the seedlings became older and the same is true for seedlings subjected to prolonged drought leading to the critical stage of wilting.

Graphs are presented to show the manner in which the water-content percentage decreased throughout the experiment period. In some cases the rate of this decrease was temporarily retarded shortly after the critical wilting stage had been passed. After this temporary retardation the rate of decrease in water-content percentage became more rapid and the seedling tops soon became brittle. The temporary retardation may correspond to the general rupture of tensile water columns and to the onset of death. It appears to have occurred when the water-content percentage had decreased by about 25 per cent of its original value for the day after the last watering.

The results obtained with soil points were remarkably consistent and satisfactory. The soil-point averages, showing the initial water-supplying power of the soil (at the 3—5 cm. depth) when the critical stage of wilting was reached in the plantlets, ranged between 11 mg. (55-day Norway pine seedlings and 24-day western yellow pine seedlings, both grown with standard watering) and 59 mg. (24-day Norway spruce seedlings grown with excessive watering). The three Norway spruce experiments on 24-day seedlings grown with standard watering gave soil-point averages of 26 mg., 27 mg., 26 mg. and the three Norway pine experiments gave corresponding averages of 18 mg., 20 mg., 19 mg. It consequently appears that the critical water-supplying power of the soil for the 3—5 cm. depth was significantly lower for the Norway pine seedlings. Older seedlings, with longer roots, did not attain the critical wilting stage until the initial water-supplying power of the soil at the 3—5 cm. depth had decreased to still lower values. On the other hand,

excessively watered 24-day seedlings of Norway spruce reached the critical wilting stage when the water-supplying power of the soil at the standard depth had decreased only to 58 mg. Deficiently watered 24-day seedlings of Norway spruce and Norway pine did not reach the critical stage until the soil-point index had decreased to 20 mg. (spruce) or 12 mg. (pine). For the single experiments with 24-day seedlings of the eight additional species, all grown with standard watering, the critical soil-point index ranged between 11 mg. (western yellow pine) and 30 mg. (white spruce).

To facilitate the interpretation of these critical soil-moisture indices it may be added, for example, that a soil-point reading of 24 mg. represents a water-supplying power of about 2 mg. for a cross-sectional area of 1 sq. cm., for the first hour. The insertion of the soil point compresses the soil considerably and the readings are consequently always somewhat too high to represent the undisturbed soil at the given depth. We may say that the 24-day Norway pine seedlings grown with standard watering did not attain the wilting stage taken to represent the onset of permanent wilting until the soil of the bed, at the 3—5-cm. depth, had become so dry as to be unable to supply water to 1 sq. cm. of absorbing root surface at a rate greater than 2 mg. per hour for the first hour of capillary contact with the advancing roots. At this stage of drying out, the soil of the bed at a depth range greater than 3—5 cm. must, of course, have had a higher supplying power than that just mentioned and at a depth range less than 3—5 cm. the supplying power must have been still lower than 2 mg. per hour. An initial water-supplying power of 2 mg. per square centimeter (24 mg. per soil point) is about one-fourth as great as the supplying power necessary to maintain growth in white clover and Kentucky blue-grass on a Baltimore lawn in summer, according to Livingston's ('28) estimate, which was based on measurements and observations made by Wilson ('27). Livingston says that when the initial water-supplying capacity falls to 4 mg. per square centimeter the grass leaves became dry and tawny within 2 or 3 days.

The most interesting numerical results of this whole study are brought together in summary form in Table XVIII, which is self-explanatory. It will be noted that only three of the nine experiments with Norway spruce seedlings (Exps. 2, 3, 6) represent 24-day seedlings with standard watering while only two represent 24-day seedlings with excessive watering. Three of the five experiments with Norway pine seedlings represent 24-day seedlings with standard watering. For each of the other sets of variables the results were derived from a single experiment. This study was undertaken mainly as an attempt to develop useful methods and as a sort of reconnaisance survey of possibilities, rather than to secure numerical results that might, in themselves, be reliable as representing the several species and the several experimental treatments. Time for a reasonable number of repetitions was lacking. It therefore seemed desirable to make the study broad rather than deep. The

TABLE XVIII. *Summary of critical values for all experiments.*

Species	Kind of watering during growing period.	Approximate age in days[1] of seedlings when watering was discontinued	Exp. No.	Average dry weight per seedling top in Mg.		Average water content per seedling top					Average initial water supplying power of soil[4] at critical wilting; Mg.
						Absolute in Mg.		As percentage on basis of dry weight			
								Actual		Relative, at critical wilting[3]	
				In health[2]	At critical wilting	In health[2]	At critical wilting	In health[2]	At critical wilting		
Norway spruce	Standard	24	2	6.3	6.7	24.1	23.1	383	340	0.89	26
			3	5.9	5.9	24.5	21.4	416	353	0.85	27
			6	5.7	5.9	23.1	18.5	411	327	0.80	26
			Ave.	**6.0**	**6.2**	**23.9**	**20.9**	**403**	**340**	**0.85**	**26**
		60	7	7.8	7.6	17.0	14.6	219	190	0.87	20
		68	8	7.9	9.0	17.0	17.0	216	185	0.86	12
		97	9	9.9	9.4	16.1	12.1	166	127	0.76	17
	Excessive	24	1	7.6	7.8	29.9	28.5	396	332	0.84	59
			4	6.0	6.3	23.9	21.8	403	346	0.86	58
			Ave.	**6.8**	**7.1**	**26.9**	**25.2**	**400**	**336**	**0.85**	**59**
	Deficient	24	5	4.7	5.6	20.6	20.0	445	338	0.76	20
Norway pine	Standard	24	10	4.9	4.8	21.5	18.4	442	383	0.87	18
			11	4.5	5.6	24.4	24.5	542	436	0.80	20
			12	4.5	5.7	22.9	22.8	510	400	0.78	19
			Ave.	**4.6**	**5.4**	**22.9**	**21.9**	**498**	**406**	**0.82**	**19**
	Deficient	24	13	4.3	4.2	23.0	24.1	551	436	0.79	12
White spruce	Standard	24	15	3.5	3.6	15.1	13.1	427	364	0.85	30

[1] Age is reckoned from time seedlings emerged from soil.
[2] "In health" refers to the observation made on the day after the final watering.
[3] The relative percentage value for healthy seedlings (the day after the final watering) is always 1.00.
[4] Water-supplying power always refers to a standard soil point (about 12 sq. cm.), to a depth of 3-5 cm. and to a 1-hour exposure of the instrument.

TABLE XVIII *(continued)*.

Species	Kind of watering during growing period.	Approximate age in days[1] of seedlings when watering was discontinued	Exp. No.	Average dry weight per seedling top in Mg.		Average water content per seedling top					Average initial water supplying power of soil[4] at critical wilting; Mg.
						Absolute in Mg.		As percentage on basis of dry weight			
								Actual		Relative, at critical wilting[3]	
				In health[2]	At critical wilting	In health[2]	At critical wilting	In health[2]	At critical wilting		
Northern white cedar..	Standard	24	16	1.6	1.6	6.0	5.4	388	322	0.83	22
Short leaf pine.....	"	"	17	6.4	7.8	34.5	30.0	514	399	0.78	12
Slash pine.....	"	"	18	16.9	12.9	89.8	39.9	537	318	0.59	13
Western yellow pine...	"	"	19	17.7	17.8	92.0	68.0	528	412	0.79	11
Western white pine...	"	"	20	9.0	12.6	41.4	37.3	457	293	0.64	20
Douglas fir.....	"	"	21	6.4	7.6	36.8	36.1	574	476	0.83	14
Lowland white fir.....	"	"	22	11.9	12.4	69.3	54.3	594	448	0.75	14
Western red cedar....	"	"	23	1.4	1.8	7.2	4.1	508	140	0.28	14
Western hemlock.....	"	"	24	1.0	1.0	5.1	2.6	531	256	0.48	25

results are of course relatively more significant for experiments performed three times or twice than for those performed only once, but in no case is it permissible to place very great reliance on the actual numerical values given. They are only suggestive and indicative at best and they refer only to the experiments from which they were derived. Still, they do show possibilities and probabilities in a general way and they may be useful in planning more thorough experimentation in this important field of seedling physiology and forest ecology.

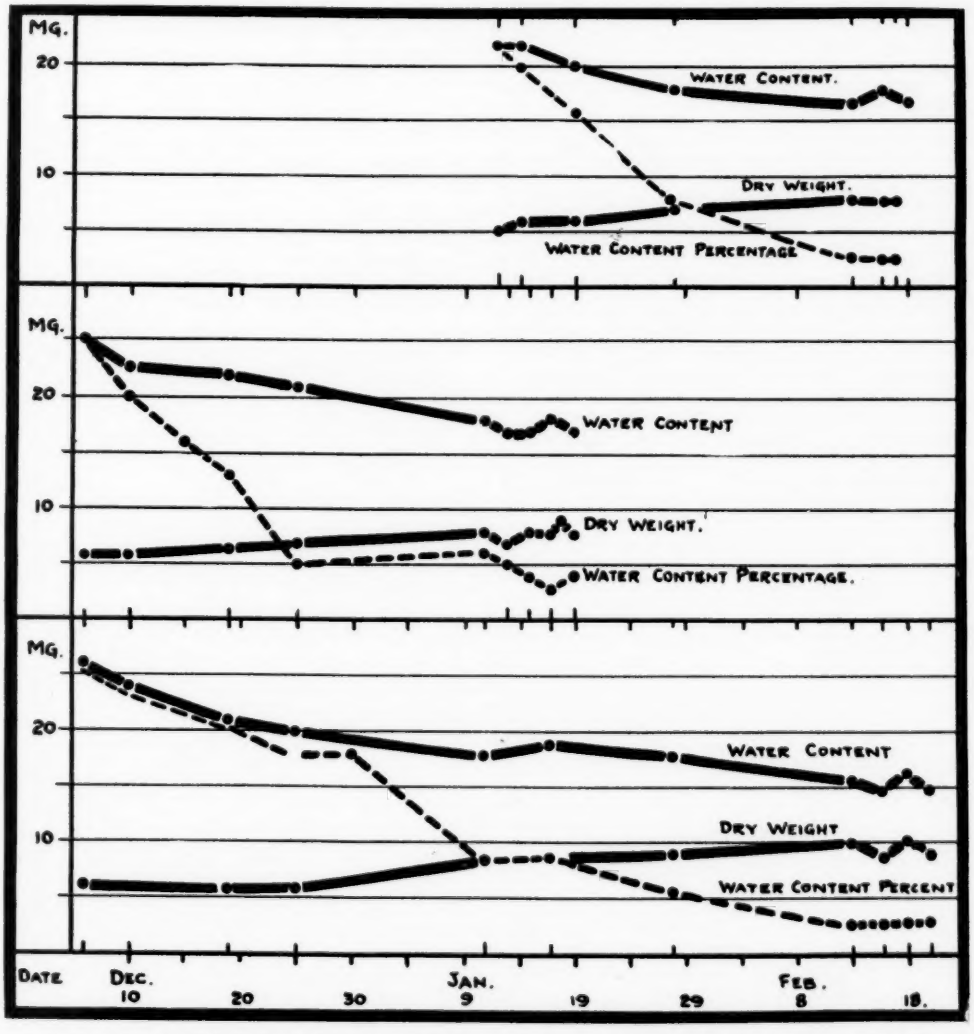

FIG. 1. Graphs showing decrease in average water content of tops and corresponding increase in average dry weight and in the water-content percentage (broken line) as Norway spruce seedlings developed. Data are from Table VIII; the three sets of graphs are for Experiments 7, 8 and 9, respectively.

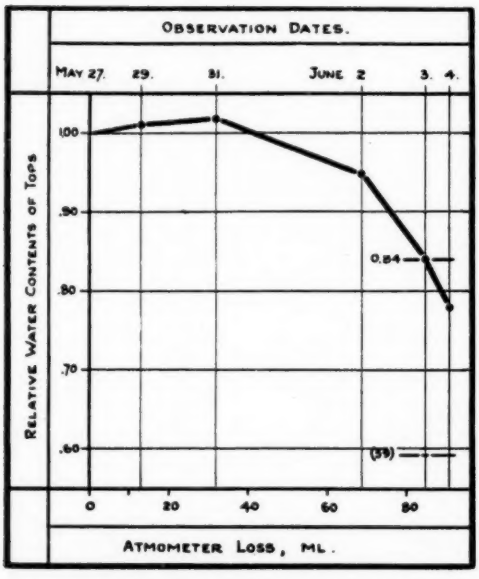

FIG. 2. Graph showing decrease in relative water content of tops of Norway spruce seedlings during Experiment 1. Data are from Table V. Ordinate 0.84 represents the critical stage of wilting and the point below marked 59 (mg.) represents the corresponding average soil-point reading, from Table X.

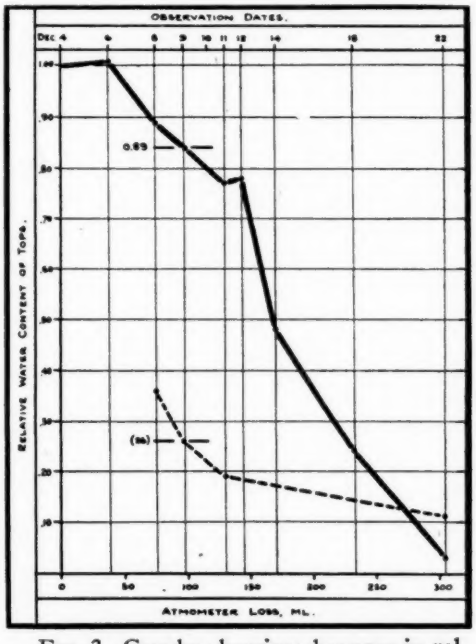

FIG. 3. Graphs showing decrease in relative water content of tops of Norway spruce seedlings during Experiment 2 (continuous line) and the corresponding decrease in average soil-point reading (broken line). Data are from Tables V and X. Ordinates for critical stage of wilting are 0.89 and 26 mg. For the broken-line graph the scale at left is to be read as whole numbers representing milligrams.

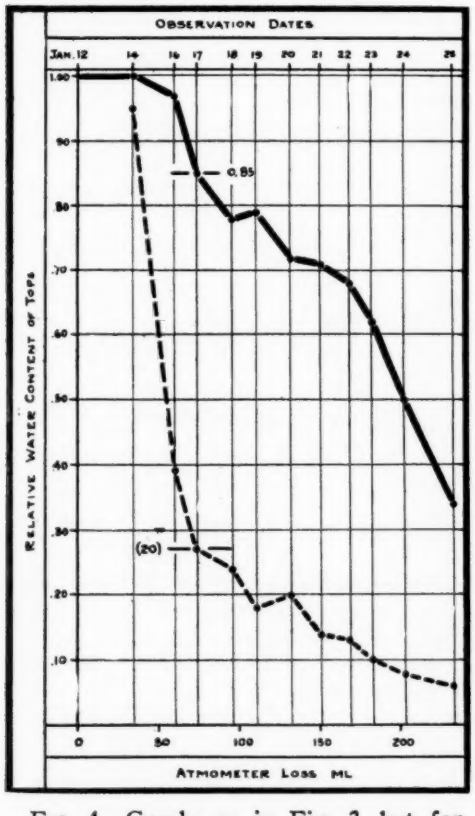

FIG. 4. Graphs as in Fig. 3, but for Experiment 3.

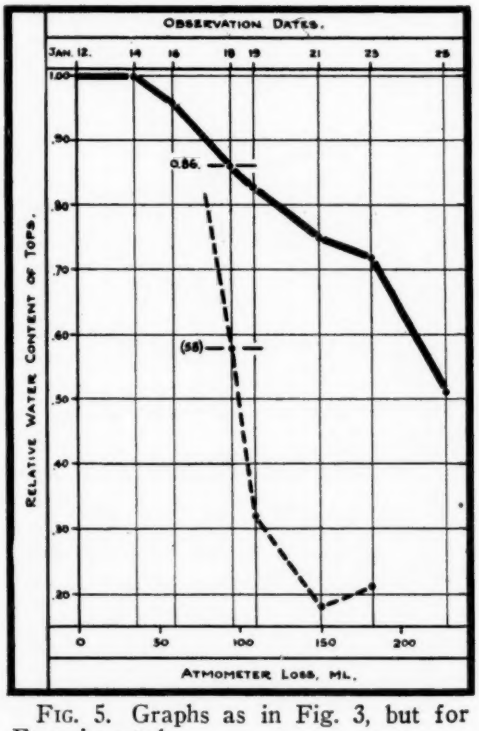

FIG. 5. Graphs as in Fig. 3, but for Experiment 4.

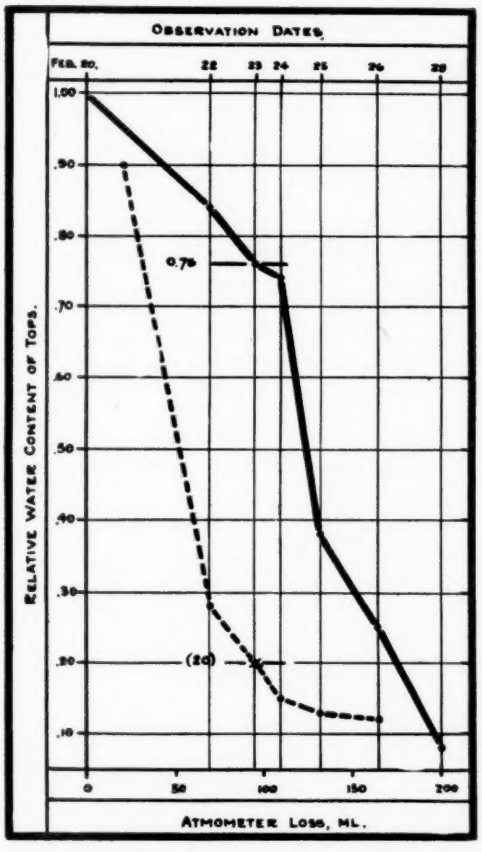

FIG. 6. Graphs as in Fig. 3, but for Experiment 5.

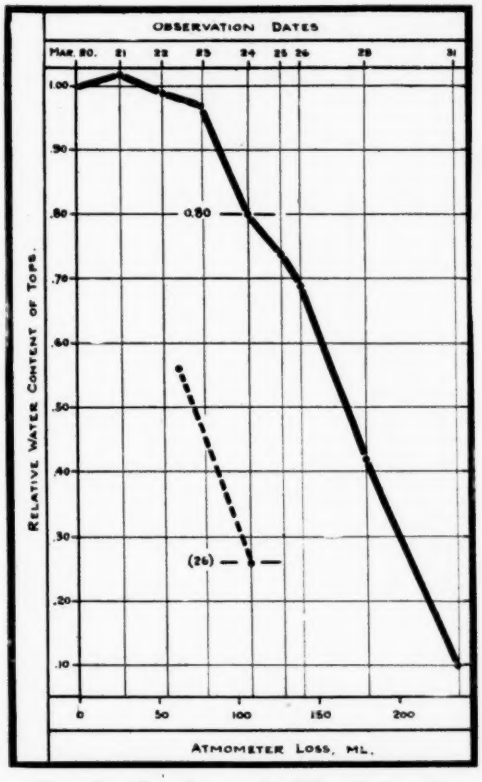

FIG. 7. Graphs as in Fig. 3, but for Experiment 6.

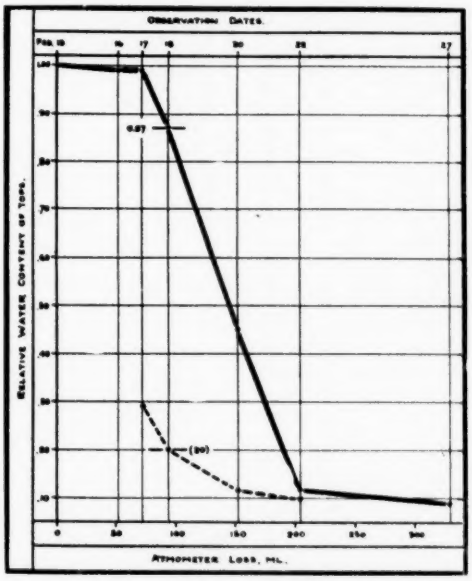

FIG. 8. Graphs as in Fig. 3, but for Experiment 7.

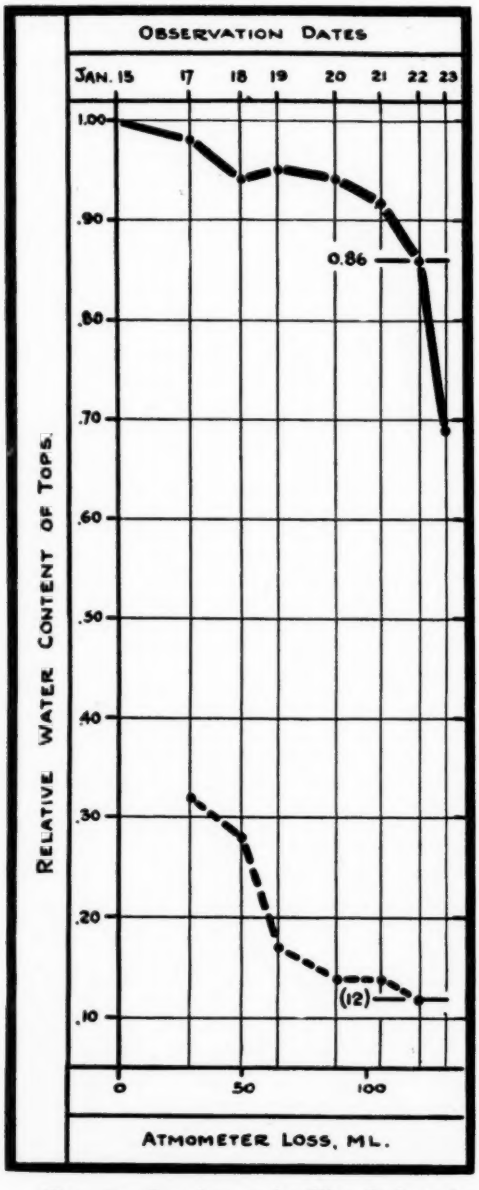

FIG. 9. Graphs as in Fig. 3, but for Experiment 8.

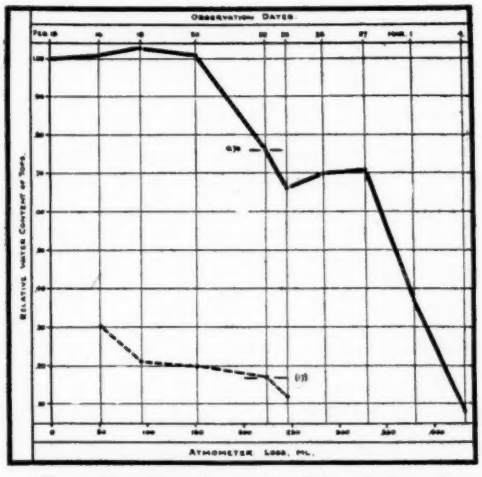

FIG. 10. Graphs as in Fig. 3, but for Experiment 9, with Norway spruce seedlings. Data are from Tables V and X.

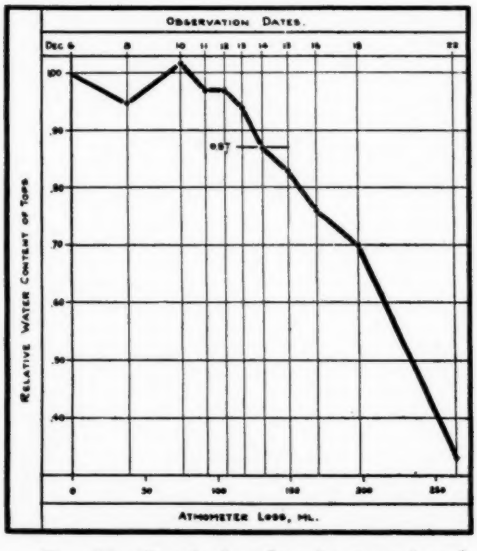

FIG. 11. Graph showing decrease in relative water content of tops of Norway pine seedlings during Experiment 10. Data are from Table XI.

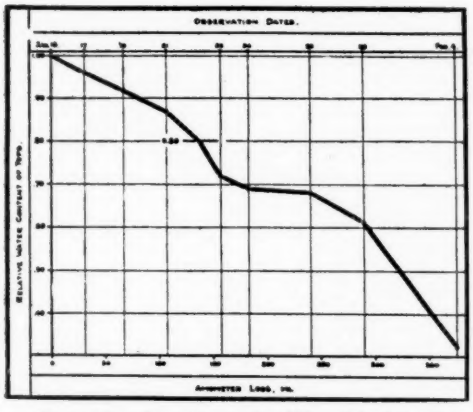

FIG. 12. Graph as in Fig. 11, but for Experiment 11.

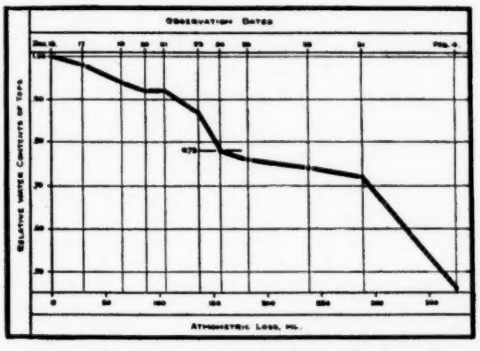

FIG. 13. Graph as in Fig. 11, but for Experiment 12.

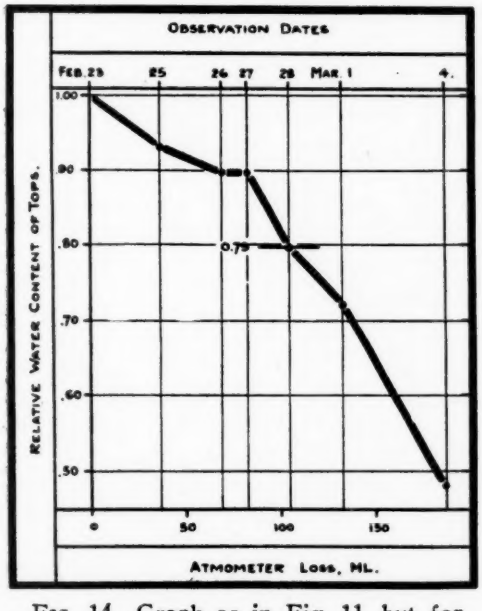

FIG. 14. Graph as in Fig. 11, but for Experiment 13.

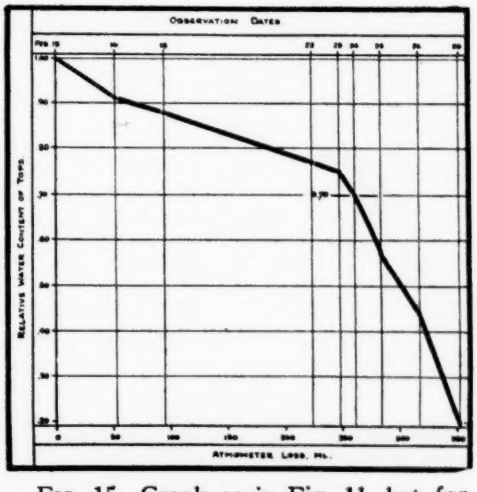

FIG. 15. Graph as in Fig. 11, but for Experiment 14.

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